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Oxandrolone Therapy: 25 Years Experience

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Background

Oxandrolone is a synthetic anabolic steroid having the chemical name 17β-hydroxy-17α-methyl-2-oxa-5α-androstan-3-one. Structurally, oxandrolone is a derivative of testosterone but is unique among all other testosterone analogues in that it contains an oxygen atom instead of a methylene group at the 2 position of the phenanthrene nucleus. The structural formula for oxandrolone is shown in Figure 1 (see page 3).

Pharmacologically, oxandrolone possesses both anabolic and androgenic activity at a ratio of approximately 6:1.[1] When taken orally, oxandrolone is rapidly absorbed and excreted primarily in the urine (approximately 25% as the parent compound). Peak plasma levels occur at approximately 45 to 90 minutes after ingestion. The biologic half-life of oxandrolone is approximately 9 hours.[2]

Human experience with oxandrolone is extensive. Although originally marketed for its anabolic activity to promote weight gain in various medical conditions, review of the published literature indicates that the primary use of oxandrolone over the past quarter century has been for the enhancement of growth velocity in children with various growth disorders (eg, constitutional delay of growth and puberty [CDGP] and Turner syndrome). Toward this end, oxandrolone has been administered to several hundred patients (age 3 to 18 years) in documented clinical trials at a typical dose of 0.1 to 0.125 mg/kg/d for up to several years. Currently, most pediatric endocrinologists recommend a maximum daily dose of 0.1 mg/kg/d or less in the treatment of Turner syndrome and CDGP. Results from published studies indicate that oxandrolone can be used effectively to increase growth velocity in girls with Turner syndrome and in boys with CDGP and that it can be used safely if the bone age is ≥ 9 years.

Overview of Oxandrolone Use in Turner Syndrome

Clinical management of Turner syndrome in childhood focuses primarily on growth therapy. Of particular importance with regard to growth is the effect a therapeutic agent has on growth velocity during treatment and on final adult height. The ideal treatment should also have a positive effect on the psychosocial status of girls with

Letter From the Editor

The protein anabolic steroid oxandrolone will again be available in mid-1991. In May of 1989, G.D. Searle & Co made a business decision to halt distribution of oxandrolone (Anavar®). Because this action adversely affected how numerous pediatric endocrinologists treat their patients, the Lawson Wilkins Pediatric Endocrine Society (LWPES) and the American Academy of Pediatrics (AAP) have encouraged Gynex, Inc and the US Food and Drug Administration (FDA) to again make oxandrolone available for the treatment of patients with Turner syndrome and constitutional delay of growth and puberty (CDGP).

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Turner syndrome (presumably by increasing growth), since many of these girls reportedly suffer from self-consciousness, embarrassment, and poor self-esteem.[3-5] Furthermore, improved psychosocial status during adolescence may yield significant long-term benefits as girls with Turner syndrome reach adulthood. A positive effect of oxandrolone on growth and psychosocial status in girls with Turner syndrome was reported by Rosenbloom and Frias in 1973.[6]

Oxandrolone alone or in combination with growth hormone (GH) has been shown to markedly enhance growth in girls with Turner syndrome. Rosenfeld et al[7] reported the results of a 3-year randomized prospective trial of methionyl human GH alone versus oxandrolone alone versus the combination in Turner syn-

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Based on an agreement reached between Gynex, Inc and the FDA, oxandrolone will be made available by 2 separate routes. The first will be under an open protocol that will be available to pediatric and adult endocrinologists with experience in the clinical management of pediatric growth disorders. The patients must meet the specific inclusion criteria for Turner syndrome, ie, having a karyotype that confirms the diagnosis of Turner syndrome, a bone age ≥9 years, a chronologic age of 10 to 16 years, and have parental informed consent and patient assent. The exclusion criteria for these Turner syndrome patients will include concurrent administration of estrogen, a history of a condition known to adversely affect growth, and/or a medical condition precluding the use of oxandrolone. In addition, the patient cannot be a ward of the state. The inclusion criteria for boys with CDGP will be a chronologic age of 11 to 16 years, a height ≤2 SD for age, a bone age ≥9 years, a delayed bone age >2 years from the chronologic age, and pubertal development of G1 or G2 and PH1 or PH2. The exclusion criteria for patients diagnosed as CDGP are GH deficiency, history of a condition known to adversely affect growth, a medical condition that precludes the use of oxandrolone, concurrent therapy with another androgen, and state wardship.

The distribution of oxandrolone will be tightly controlled by: (*a*) verifying each physician's credentials through a physician registration program, which includes a physician review by a national institutional review board (IRB); (*b*) confirming that patients are eligible for oxandrolone therapy prior to shipment of drugs; (c) closely monitoring drug use; (*d*) halting distribution if the patient is noncompliant; and (*e*) shipping oxandrolone directly to the patient (or physician, if required by state regulations) from a single distribution center. Patients for whom oxandrolone is prescribed under the open-label studies must pay for their prescription. The cost for oxandrolone will be higher than in the past; however, it is important to note that the cost established will be based on a cost recovery program regulated by the FDA. Protocols and informed consent documents for the open-label protocols have been reviewed and approved by a national IRB established by Gynex, Inc in cooperation with the LWPES and the AAP. Members of the IRB are Jose Cara, MD, Wyler Children's Hospital, University of Chicago; Nancy Hopwood, MD, Children's Hospital, University of Michigan; Edward Reiter, MD, Baystate Medical Center, Springfield, Massachusetts; S. Douglas Frasier, MD, Olive View Medical Center, Los Angeles County; Lynn Georgia-Tesch, JD, Turner Syndrome Society; and William D. Stout, lay member. Physicians not constrained by local IRB policies may participate in the open-label use of oxandrolone through the national IRB.

As a part of the agreement between Gynex, Inc and the FDA, the second available route for obtaining oxandrolone involves patient participation in Phase III placebo-controlled clinical trials either in girls with Turner syndrome or in boys with CDGP. Subjects enrolled in either of these control studies will receive oxandrolone free of charge. Those patients who are randomized to the control group will (upon the recommendation of the physician) be eligible to receive up to a 12-month supply of oxandrolone for free following their participation in the clinical trial. The placebo-controlled study in Turner syndrome will be supervised by JoAnne Brasel, MD, Harbor Hospital/UCLA Medical Center, Torrence, California. The placebo-controlled study in boys with CDGP will be supervised by Darrel Wilson, MD, Stanford University School of Medicine, Stanford, California. Forty subjects (n=20/group) are needed for each 12-month study.

Your support of the studies discussed above will have a *direct impact* on the long-term availability of oxandrolone. ***Information about these studies, physician registration, and the distribution programs are available from Gynex, Inc (708-913-7708).***

Robert M. Blizzard, MD — Editor

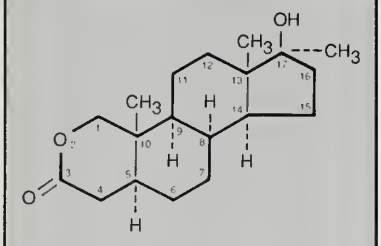

Figure 1: Structural Formula Of Oxandrolone

drome. After 12 to 20 months of therapy, the growth velocity of girls treated with 0.125 mg/kg/d oxandrolone alone was significantly greater than controls (7.6 ± 1.5 cm/yr versus 3.8 ± 1.1 cm/yr) and statistically equivalent to the growth velocity observed in girls treated with GH alone (6.6 ± 1.2 cm/yr). Of perhaps greater significance, the combination of oxandrolone and GH yielded a growth rate of 9.8 ± 1.4 cm/yr, suggesting a synergistic action of the drugs on growth velocity. These findings are consistent with earlier work[8] and with more recent findings.[9] Although the effect of combination treatment on final adult height is yet to be clearly defined, these results suggest that combination therapy may yield better results than either treatment alone for Turner syndrome.

Oxandrolone alone has been shown to increase growth velocity and, in some cases, final adult height of girls with Turner syndrome. Rosenbloom and Frias[6] treated girls 9 to 18 years of age with approximately 0.1 mg/kg/d oxandrolone for 4 to 36 months. Oxandrolone significantly increased growth velocity from a mean pretreatment rate of 1.8 cm/yr to an average of 5.3 cm/yr over the 4- to 36-month period of treatment. For the 7 girls treated more than 1 year (average, 22 months), the mean bone age advance was 9 months. Moore et al[10] reported similar results and reported an increase in mean final adult height in 9 of 20 girls treated with oxandrolone. Stahnke et al[11] studied the effects of oxandrolone in girls (mean age of 14 years) treated with 0.1 mg/kg/d for 1.5 to 6 years. Significant increases in growth velocity were observed that tended to decline over time (eg, to 2.1 cm/yr after 5 years). Notably, oxandrolone therapy increased final adult height in many patients, a finding also reported by Heidemann et al.[12] Finally, Joss and Zuppinger[13] conducted the only pair-matched controlled study of oxandrolone in which the patients were studied to final height. Patients received oxandrolone (0.1 mg/kg/d) for either 1- or 2-year treatment periods (with a 6-month interval off therapy). Oxandrolone led to a marked increase in height velocity from a pretreatment value of 2.9 cm/yr to 5.0 cm/yr during the first year of therapy. Figure 2 provides a graphic example of the positive effect of oxandrolone on growth velocity as expressed in standard deviations (SD) in patients with Turner syndrome.[13]

Fifteen of the 20 patients treated with oxandrolone reached final adult height (n=7 for 1 year of therapy and

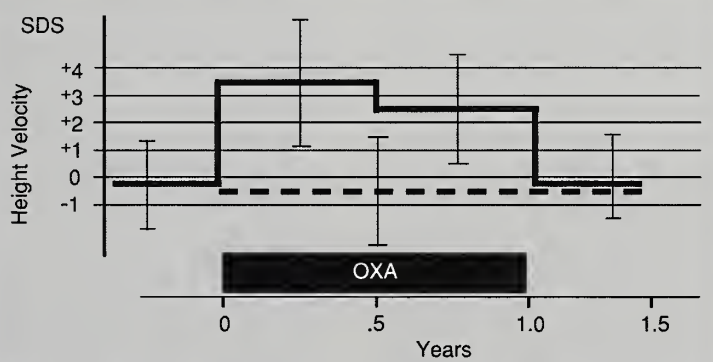

Figure 2: Height velocity (SDS) in patients before, on, and after oxandrolone in a dose of 0.1 mg/kg/day (*full line*) (*n*=26) compared to the height velocity of untreated matched controls (*broken line*) (*n*=26). Mean ±1 SD.

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n=8 for 2 years). In this study, final adult height was significantly increased in oxandrolone-treated patients compared with untreated matched controls.

A noteworthy aspect of the studies discussed above is the absence of significant side effects in response to oxandrolone therapy. At a dose of 0.1 mg/kg/d, most girls treated with oxandrolone did not experience acne, deepening of the voice, growth of facial hair, or clitoromegaly. Occasional notations have been made in the literature regarding small, transient elevations in liver transaminases in some girls with Turner syndrome treated with oxandrolone. Impaired glucose tolerance (ie, insulin resistance) in some girls treated with oxandrolone (0.125 mg/kg/d) alone or in combination with GH for 12 months has been reported.[14] The clinical importance of this observation could not be determined in light of the fact that both fasting glucose and glycosylated hemoglobin concentrations remained normal. In addition, glucosuria was not observed in oxandrolone-treated girls. Thus, short-term complications from impaired glucose tolerance seem unlikely; and although potential long-term effects of oxandrolone-induced impaired glucose tolerance in girls with Turner syndrome are unknown, it is noteworthy that no serious adverse effects related to glucose metabolism have been reported in girls treated at higher oxandrolone doses for substantially longer periods of time.

Oxandrolone, like all androgens and estrogens, can cause premature skeletal maturation if inappropriately large dosages are administered to young patients. At a daily dosage of 0.1 mg/kg or less, oxandrolone has generally not been associated with inappropriate aging of bone provided treatment is withheld from girls with a bone age of less than 8 to 9 years.[13,15,16]

Therefore, the collective experience with oxandrolone in the treatment of Turner syndrome indicates that oxandrolone can be safely and effectively used to increase growth velocity. The effect of oxandrolone treatment on final adult height in Turner syndrome is somewhat controversial, but at low doses (ie, 0.1 mg/kg/d or less) and in girls with bone ages of ≥8 to 9 years at initiation of therapy, there are data to suggest that final adult height can be increased or, at a minimum, not adversely affected. In the latter case, oxandrolone therapy is still likely to be beneficial in that increased growth velocity often is psychologically beneficial for girls with Turner syndrome since oxandrolone therapy will stimulate linear growth at an age when normal peers are undergoing a pubertal growth spurt.

Certainly, optimal treatment of Turner syndrome would require both oxandrolone and GH earlier in childhood and probably low-dose estrogen in the late childhood years, with a suitable increase to induce secondary sexual characteristics after the age of 11 years. It is certain that all 3 therapeutic modalities are synergistic for growth in Turner syndrome but the age of commencement, the dosing regimen, and duration of treatment have not yet been determined. Certainly, oxandrolone has a significant role to play in the modern management of short stature due to Turner syndrome.

Overview of Oxandrolone Use in Constitutional Delay of Growth and Puberty

CDGP is diagnosed in otherwise healthy adolescents when height is significantly reduced for chronologic age (eg, 2 or more standard deviations below the 50th percentile) but generally appropriate for pubertal development and bone age — both of which are usually delayed.[17] The condition is often associated with a family history of delayed puberty and is reported more commonly in boys than in girls. Because the pubertal growth spurt in boys occurs when a testicular volume of 10 to 12 cc (G4) is reached, the time before a spontaneous increase in growth velocity occurs in boys with CDGP may be considerable. In addition, the growth acceleration is often blunted, which may result in a slightly lower than predicted adult height.[18] Depending on the emotional stability of the individual and his social setting, CDGP can give rise to extreme distress and may result in severe psychosocial problems.[17-22] Thus, the goal of oxandrolone therapy is to increase growth velocity and thereby improve psychosocial status in boys with CDGP.

Clinical management of CDGP depends largely upon individual patient needs. Although counseling and reassurance that growth and pubertal development will eventually occur may prove sufficient for many boys with CDGP, it may not be sufficient for others, who would thus derive substantial benefit from increased growth velocity in response to drug therapy. The latter is particularly relevant for patients experiencing psychosocial problems related to their delayed development since these may negatively impact future adult behavior. Oxandrolone has been used successfully for many years in the clinical management of CDGP.[23-30] Despite the availability of other therapeutic agents for CDGP (eg, testosterone, fluoxymesterone, GH) Stanhope et al[25] argue that

oxandrolone may represent the treatment of choice based on its (*a*) long history of use (albeit not yet approved by the FDA for this indication); (*b*) effectiveness in increasing growth velocity without adversely advancing bone age; (*c*) low incidence of side effects at doses currently reported in the published literature for clinical management of CDGP; (*d*) route of administration (oral versus intramuscular injection of testosterone, testosterone analogues, and GH); and (*e*) cost (compared with, for example, GH).

The use of oxandrolone to treat CDGP has been well documented in the medical literature and spans a 25-year period. In these studies, oxandrolone was administered at typical doses of 0.1 to 0.125 mg/kg/d to patients (n>350) generally between the ages of 8 and 17 years. Although the average duration of treatment was 3 to 12 months, some children were treated with oxandrolone for substantially longer periods (eg, up to 60 months) and at daily doses as high as 2 mg/kg. With rare exceptions, oxandrolone safely and effectively increased growth velocity[10,17,23-38] and when evaluated, improved psychosocial status. The lone exception to these findings was reported by Marti-Henneberg et al in 1975.[39] In their study, oxandrolone was without effect on growth velocity in 9 boys (11.2 to 13.3 years of age) treated with 0.1 mg/kg/d for up to 60 months. A recent (1990) study published by Buyukgebiz et al[27] comparing oxandrolone and rGH therapy showed that while both treatments resulted in significant increases in height velocity, the increment in height velocity induced by oxandrolone at 2.5 mg/d for 3 months, but observed for 1 year, was greater than GH alone at 7.7 mg (20 units)/m^2/wk for 1 year in increasing growth velocity in boys with CDGP.

A brief review of 3 recent studies with oxandrolone in boys with CDGP provides further insight into the clinical usefulness of oxandrolone. Joss et al[26] treated 27 boys (10.6 to 11.5 years old) with 0.12 to 0.22 mg/kg/d oxandrolone for 12 months and followed them until they reached adult height. While receiving oxandrolone, the mean height velocity of the boys increased 107% to 115% (ie, approximately 4.1 to 8.7 cm/yr). The mean ratios of change in bone age to change in chronologic age were 2.0 and 2.3, respectively, in boys treated with 0.12 and 0.22 mg/kg/d oxandrolone compared with a ratio of 0.9 for untreated controls. Joss et al[26] concluded that although oxandrolone did not improve final adult height, it has therapeutic value in the treatment of CDGP by increasing growth velocity and stimulating an earlier onset of puberty — the occurrence of which may benefit boys suffering from psychologic problems due to delay of growth and development. Also of significant importance was Joss's finding that use of oxandrolone did not compromise final adult stature.

Stanhope et al[25] conducted a double-blind, placebo-controlled trial of low-dose oxandrolone in CDGP. Nineteen boys with CDGP (mean age, 14.4 years) were randomized to a control or treatment (0.072 mg/kg/d oxandrolone) group. Treatment duration was 3 months. Oxandrolone-treated boys exhibited a significant increase in growth velocity (4.5 to 9.6 cm/yr). Despite cessation of treatment, growth velocity in oxandrolone-treated boys was sustained at a mean of 8.6 cm/yr over an additional 3-month period.

Tse et al[30] treated 40 boys (median age of 14.2 years) with oxandrolone at the low-dose of 1.25 mg or 2.5 mg per day. Twenty-six subjects received treatment for 3 months,12 for 6 months, and 1 each for 9 and 12 months. There was a significant increase in growth velocity, and all final heights were within the 95% confidence limits of predicted heights by the Tanner-Whitehouse II (TW2) method. The mean final height was 167.3 ± 6.6 cm versus 165.8 ± 5.9 cm predicted height (P=0.03), indicating there was no compromise in final height.

The method of treatment, as given in various reports by British investigators, differs from that used in the United States. In the latter, oxandrolone has been used on a continuing basis in boys with CDGP until testosterone and/or sexual maturity (testicular size of >10 to 12 cc volume) occurs. The British groups frequently have used only 3 months of treatment and noted that the increased growth velocity induced by oxandrolone persists with discontinuation of the agent if the testes are >4 cc in volume.[23-25,30] They report that in boys who are prepubertal (testes <4 cc) there is a growth spurt while the patient receives oxandrolone, but there is no sustained growth with discontinuation of therapy.

The mechanism of increased growth remains controversial. The studies of Link et al[31] indicated no significant increase in growth hormone production in 10 boys with CDGP with 3 months of oxandrolone therapy (approximately 0.1 mg/kg/d). Also, there was no significant increase in insulin-like growth factor I (IGF-I) levels. IGF-I levels were found to increase modestly in several reports. For example, in 1 report,[25] the mean serum IGF-I concentration increased from 1.01 U/mL to 1.23 U/mL (P <0.05). Clayton et al[24] reported

that GH concentrations during sleep did not change in prepubertal boys receiving 2.5 mg/kg/d of oxandrolone although in pubertal boys an increase was reported. Loche et al[29] and Stanhope et al[28] observed that oxandrolone increased GH secretion. Ulloa-Aquirre et al[40] reported that the mean GH production increased in 5 boys treated with oxandrolone 1.25 mg tiw. However, the increased production occurred primarily in 1 of the 5, increasing 250%, while the increase in the other 4 was marginal and, therefore, difficult to interpret. The differences in ages, total doses, time intervals between doses, and length of time between last dose and measurement of integrated GH concentration may account for the controversial reports. Further studies are indicated, such as dose response curves, to evaluate the extent to which oxandrolone stimulates growth directly and the extent to which it results in increased GH secretion.

Thus, based on the published literature, collective experience with oxandrolone indicates that it can be safely and effectively used to increase growth velocity and, although less well documented, improve psychosocial status in boys with CDGP. It should be noted that treatment of CDGP with oxandrolone advances the timing of the growth spurt with little or no interference in the rate of sexual maturation.[25,30]

References available upon request.

Obesity in Childhood and Adolescence

Part 1: Physiology, Genetics, and Growth

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Introduction

Childhood obesity is a multifactorial disease resulting from an imbalance of energy intake and expenditure. Environmental and hereditary factors play a role in the development of obesity. Environmental factors particularly contribute to increased food (energy) intake and to expenditure of energy through activity. In this review, we will consider the physiology of obesity, the evidence that genetic factors operate to produce obesity, how these factors may be expressed, and how growth may be affected in obesity.

Physiology

An individual is in energy balance when energy intake equals energy expenditure. When energy intake exceeds expenditure, the storage of body fat increases. Conversely, when energy intake is lower than expenditure, the depots of body fat decrease. Relatively small excesses in energy intake that are maintained for long periods produce significant increases in body fat. For example, an excess energy intake of 100 cal/d for a year results in 10 pounds of accumulated fat.

Food (or energy) intakes have been reported to be comparable among obese and nonobese adults,[1,2] thus suggesting that obese individuals have a reduced energy expenditure. However, others have reported that in obese individuals energy intake is significantly lower than energy expenditure, which casts doubt on the reliability of dietary records to provide a valid measure of energy expenditure.[3]

Daily total energy expenditure (TEE) is calculated based on 4 components: (1) the basal metabolic rate (BMR); (2) the thermic effect of food (TEF); (3) the energy spent in physical activity (E_A); and (4) the energy required for growth (E_G). Under normal circumstances in adolescence, the BMR accounts for 55% to 60% of TEE, TEF for approximately 10% of TEE, and E_A for approximately 25% of TEE; E_G is extremely variable according to growth velocity and/or replacement of tissue. Heredity *may* produce obesity by decreasing TEE through decreased BMR and/or decreased TEF and, possibly, through the energy necessary for activity (E_A) and growth (E_G).

TEE can be determined by the doubly labeled water method ($^2H_2{}^{18}O$) described by Schoeller.[4] Because ^{18}O is lost as both water and carbon dioxide (CO_2) and ^2H is lost as water, the differential loss of the 2 isotopes from body water over time is a measure of the rate of CO_2 production. With the knowledge of the food quotient of the diet, TEE can be measured within 5% of that determined by respiratory gas exchange. This method is ideal for children and adolescents because no equipment or confinement is necessary.

BMR can be measured continuously using indirect calorimetry with a ventilated hood.[5] Calculations are made from measures of oxygen consumption and CO_2 production according to the modified Weir's formula.[6] In

adolescents, BMR accounts for 55% to 60% of TEE. The BMR is affected by fat-free mass (FFM), fat mass, age, stage of sexual development, and familial characteristics. However, the *principal determinant* of the BMR is FFM. Increase in Tanner staging and in age improve the correlation between the BMR and the FFM ($r = 0.93$) in adolescents.[7]

A different relationship between FFM and BMR has been shown for males and females and obese and nonobese adolescents, which suggests that *both* sex and fat mass contribute to the variability in the BMR.[7] Bogardus et al reported that familial characteristics also contributed significantly to the BMR in a group of Southwestern Indians.[8] Presumably, the same could occur in other family groups.

Because the BMR contributes significantly to the total metabolic rate, decreases in BMR will reduce total energy needs. In a study of obese and nonobese adolescents,[7] the BMR adjusted for differences in body composition was increased in the obese group (Table 1). These findings suggest that the normal obese adolescent does not have a reduction in metabolic rate. Bogardus et al[8] found no significant differences in fat mass in individuals from families with high and low metabolic rates.

Although a reduction in BMR does not seem to be a factor in the *maintenance* of adolescent obesity, it theoretically could contribute to the *development* of obesity.

Prospective studies by Ravussin et al[9] in adults suggest a significant relationship between TEE and weight gain. Specifically, Ravussin et al demonstrated greater weight gains in those Pima Indians who had low adjusted BMRs and TEEs. Following weight gain the metabolic rates increased. These data suggest that individuals with a low metabolic rate may gain weight as a compensatory mechanism to normalize the BMR and increase energy expenditure.

The TEF is reflected in the rise in metabolic rate after eating. This increase in energy expenditure is the energy necessary to process the food. The TEF has a genetic component[5,10] and contributes approximately 10% to the TEE. Small decreases in the TEF over a prolonged period of time could lead to a significant energy imbalance and an increase in body fat stores. Therefore, significant attention has recently focused on the TEF. However, these studies, which were performed primarily in adults, are inconclusive. Some studies reported a reduced TEF in the obese while others did not. However, there were significant differences in study designs, nutrients ingested, caloric content, criteria for obesity, heterogeneity of the subjects, and duration of the studies. For example, some investigators fed similar amounts of calories to obese and nonobese subjects, while others based the caloric intake on body weight, FFM, or a percent of BMR. Some of the differences in outcomes can be attributed to the altered body composition in obese subjects. Segal et al[11] controlled many of these variables by matching obese and nonobese subjects for FFM. Their results indicate that there is a blunted TEF in the obese. However, we were unable to demonstrate significant differences in the TEF in obese and nonobese adolescents, although FFM was similar in the 2 groups.[5]

E_A is the most variable component of energy expenditure. E_A can be calculated if TEE, BMR, and TEF are known by using the formula E_A

Table 1
Fat-Free Mass and Energy Expenditure in Obese and Nonobese Adolescents†

	NONOBESE		OBESE		SIGNIFICANCE*	
	Females	Males	Females	Males	Females	Males
FFM (kg)	40.9	47.1	52.6	55.9	-	-
BMR (kcal/d)	1,441	1,742	1,918	2,253	yes	yes
TEE (kcal/d)	2,385	3,109	3,282	3,612	yes	yes
TEE-BMR+	944	1,367	1,364	1,359	yes	no
TEE/BMR-	1.69	1.79	1.68	1.68	no	no

\+ Nonbasal energy expenditure (direct calculation)
\- Nonbasal energy expenditure (relative or indirect calculation)
* Obese vs nonobese
† Table modified from reference number 7
FFM, fat-free mass: BMR, basal metabolic rate; TEE, total energy expenditure.

= TEE -(E_{BMR} + E_{TEF}). The energy costs of growth (E_G) are very small and are considered negligible in this calculation. Another calculation that reflects E_A is the ratio TEE:BMR, which reflects the amount of energy spent above the BMR. The ratio of TEE: BMR did not differ significantly between obese and nonobese adolescents in our study,[7] although TEE was greater in the obese group (See Table 1, page 7). These results indicated that the proportion of E_A and TEF was not reduced in the obese groups. However, a significant reciprocal relationship existed between nonbasal energy expenditure and body fat,[7] suggesting that the amount of energy spent above basal level decreases with increased body fat and that obese and nonobese individuals are not equally active. Because an increase in body size requires an increased amount of energy be spent in performing the same physical activity, the overall or total physical activity level of obese individuals may be lower than that of comparable nonobese individuals. This finding supports the previously reported work by Bullen et al[12] who found obese girls to be less active than nonobese girls. Together, these data suggest that the obese adolescent is less active overall, although the energy spent in performing similar activities may be relatively equivalent. These observations are supported by studies of infants which demonstrate excess weight gain despite unaltered metabolic rates, when a lower TEE exists, ie, the infants who gained the most weight had decreased levels of physical activity.[13]

There is a theory that some individuals are able to overeat but burn the excess calories as heat, while others are more energy efficient and store the excess calories as fat. This concept has been termed *facultative thermogenesis* or luxus consumption. In obese adolescents in whom BMR, TEF, and TEE were measured during a maintenance period and after 2 weeks of overfeeding, the thermogenic response to overeating was not reduced.[5] Additionally, the majority of overfeeding studies in which energy expenditure was measured do not support a role for facultative thermogenesis in the maintenance of body weight.[14-17]

Genetics

Obesity occurs with a greater prevalence among children with 2 obese parents than among those with 1 obese parent or no obese parents.[19] Although studies of the resemblance in fatness between pets and their owners suggest a strong environmental component,[20] genetic factors play an important role. This has been demonstrated in studies of subcutaneous fatness, as determined from measures of skin-fold thickness of twins. Bouchard et al[21] examined subcutaneous skin-folds in adopted and biologic siblings, cousins, and monozygotic (MZ) and dizygotic (DZ) twins. The intraclass pair correlations were highest for MZ twins (r = 0.76 to 0.87), followed by DZ twins (0.30 to 0.49), biologic siblings (0.18 to 0.43), and cousins (0.21 to 0.29). The intraclass correlations for pairs of adopted siblings or unrelated siblings were essentially zero.

Because a similar environment existed for these twins, it is difficult to determine the genetic contribution in fat accumulation. More recently, definitive studies to determine the heritability of fatness and obesity have focused on MZ and DZ twins living in similar or dissimilar environments or on adoptees separated from biologic parents. For these studies, body mass index (BMI) (weight in kilograms/ body surface area in square meters), which is strongly correlated with body fatness in adults, was used as the parameter to determine the heritability of fatness. In both Stunkard et al's twin study[22] and adoption study,[23] heredity appeared to be a major determinant of BMI. In the adoption study, Stunkard et al found a strong relationship between the BMIs of Danish adoptees with the BMIs of their biologic parents, but not with the BMIs of their adoptive parents.

These findings were interpreted to suggest that childhood family environment alone has little or no effect on the development of obesity. Careful inspection of the data, however, suggested that the significance of the BMI relationship between adoptees and their biologic parents resulted from the resemblance of the BMIs of lean adoptees and their biologic parents.[24] No significant difference in the prevalence of obesity existed between obese and overweight adoptees and either their adoptive or biologic parents.

In a more recent (1990) study, Stunkard et al[25] compared the BMIs of Swedish adult MZ and DZ twins reared together or apart. The mean age of the population was 58 years but few members of the study were obese. Intrapair BMI correlations were 0.66 to 0.77 in MZ twins reared apart and were comparable to the correlation observed in twins reared together (0.66 to 0.74). Although the authors concluded that the childhood environment has little or no influence on BMI, these findings offer limited insight into the heritability of obesity. As stated previously, BMI is an indirect measure of body fatness. Therefore, similarities in members of a nonobese population may reflect that the

size of the body frame rather than obesity is inherited. Moreover, this study was limited to Scandinavia, where similar lifestyles may have minimized the environmental contribution to fatness in twins reared apart.

Recently, Bouchard and coworkers overfed 6 pairs of MZ twins for 100 days to elucidate the role of the genetic component on the storage of energy.[18] Although weight gain and body fat distribution were more similar within twin pairs than between twins, the intrapair correlation coefficient was approximately 0.5. This indicated that a significant portion of the variance in weight gain and fat distribution was unexplained by genetics. Since TEE was not measured, it is unclear whether the energy cost of fat accretion differed more between than within twin pairs. Some twin pairs apparently were more energy efficient and unmeasured differences in E_A may have contributed to the variability.

We conclude that the relative contributions of genetic and environmental factors to the energy imbalance that produces childhood obesity are as yet unclear. Obesity is clearly related to genetic factors, but published studies have been confounded by environmental factors and failure to distinguish frame size from fatness. Lack of differences in energy expenditure between obese and nonobese adolescents does not exclude the possibility that before becoming obese, the obese child had a reduced energy requirement. The next major challenge for research in obesity is to demonstrate to what extent reductions in BMR, TEF, activity, and/or daily energy expenditure are genetically mediated and, therefore, increase the susceptibility to obesity.

Growth

Clinically obese children tend to be taller and to demonstrate greater maturational advancement than their nonobese counterparts. Fatter children are both larger in body size and advanced in skeletal maturation, as reported in a review of the literature by Garn et al in 1973.[26] Lean (<15th percentile for triceps skin-fold measurements) and obese (>15th percentile) children were separated out of a group, and their heights were analyzed. The obese children were significantly taller (by as much as 6 cm or more) than the lean children. The lean boys and girls averaged -0.21 Z scores or 0.2 SD below stature expectancy while the obese children averaged 0.48 Z scores or 0.48 SD above height expectancy, as calculated on the basis of the total 4,888 children studied. By the ages of 11 and 12 years, the lean children were nearly 0.4 SD below the median and the obese children were nearly 0.6 SD above the median, with a difference of nearly 1 SD between the groups. The lean boys and girls were below the median at all ages considered, and the obese children were above the median at all ages. Appropriately, the authors did not conclude whether obesity was prone to produce accelerated growth or whether children with accelerated growth were more prone to be obese.

Forbes[27] reported that obese children who became obese during infancy tended on average to have a greater relative height than those who became obese in childhood. Subsequently, using data collected in a longitudinal growth study, Forbes reported that children who developed obesity during childhood reveal a distinct tendency for height to accelerate coincident with or after the onset of excessive weight gain.

The magnitude of the relative height increment is related to the degree of overweight. "Overnutrition accelerates growth just as undernutrition retards it."[27]

Recently, Vignolo et al reported a study on growth and development in obesity in 303 subjects.[29] Obesity was defined as a weight >20% than that expected for height and sex. Adiposity strongly correlated with BMI and skin-fold measurements. Twenty-five percent of boys and 29% of girls were above the 90th percentile for height when first seen. As they approached adolescence, they moved closer to, and then below, average stature.

Thus, although prepubertal children who are obese are taller than their peers, the data are conflicting as to whether these children remain taller by adolescence.

Growth velocities (GVs) decrease during weight reduction. We demonstrated that even mildly restrictive diets may be associated with a reduction in linear growth velocity.[28] In 19 children studied, the mean SD score for GV was 2.3 ± 2.4 prior to weight reduction, which is in accord with the relationship between obesity and increased height. For the 11 patients with GVs >2 Z scores above the mean, the mean Z score decreased significantly to 0.62 ± 2.37 on a restrictive diet. The data did little to identify the cause of the reduction in GV, and further research in this field is very much indicated. Regardless, the data emphasize the need for careful monitoring of GVs of obese children during weight reduction.

The hormonal and nutritional biochemistry that produces a correlation of increased growth and maturation in obesity remains to be unraveled, but offers a fertile field for investigation.

References available upon request.

Final Height in Turner's Syndrome and Effect of Oxandrolone

Two separate papers published simultaneously by a group from Denmark provide new data for growth studies and therapeutic trials in Turner syndrome. The first paper compares the results of different methods for predicting final height in 20 Turner girls aged 9.5 to 18 years, also incorporating data from 78 adult Turner women in the same geographic area. This allowed the researchers to calculate an index of potential height (IPH) appropriate to Turner syndrome that yielded more accurate adult height predictions than those, usually overestimated, obtained using either Bayley-Pinneau or Tanner-Whitehouse II methods. Combination of the IPH with 1 of these 2 bone age-based methods yielded the greatest accuracy.

The second paper reports the effects of oxandrolone (0.125 mg/kg/d for 2 years) in 32 Turner girls aged 11.5 to 16.7 years at the onset of treatment. The results were calculated by comparison with the Danish Turner standards mentioned above, and showed a significant increase in growth velocity in the patients aged <13 years. Twenty-two patients have reached their final height and a significant ($P<0.001$) improvement of 3 to 4 cm over the height predicted by the IPH-bone age method was obtained in those with an initial bone age below 13 years, but not in the others. At this dosage level, oxandrolone produced mild androgenic side effects in 5 patients that were reversible in all cases (except in 1 girl with slight deepening of the voice).

Naeraa RW, Eiken M, Legarth EG, et al. Prediction of final height in Turner's syndrome: a comparative study. *Acta Paediatr Scand* 1990;79:776-783.

Naeraa RW, Nielsen J, Pedersen IL, et al. Effect of oxandrolone on growth and final height in Turner's syndrome. *Acta Paediatr Scand* 1990;79:784-789.

Editor's comment: *Once again, these papers show how difficult it is to make height predictions in patients with Turner syndrome. The IPH calculation proposed has been, in the authors' hands, better than the usual Bayley-Pinneau, Tanner-Whitehouse II or Roche-Wainer-Thissen methods. However, it is complicated and depends on data from untreated Turner adults in the same locale, so that it is probably more appropriate for large-scale scientific studies than for daily clinical use. The application of the IPH method here to evaluate the end results obtained with oxandrolone is a good example. Regarding treatment with oxandrolone, it must be stressed that good results were limited to girls with a bone age of 10.0 to 12.9 years treated over 2 years with a relatively high dose of oxandrolone, which resulted in androgenic side effects in 16% of the girls treated. Thus, only in this sense does it seem that the data will contribute to a better evaluation of the end results to be obtained in Turner patients with growth hormone alone or with the growth combined with low-dose androgens.*

Jean-Claude Job, MD

2nd Editor's comment: *The primary importance of these studies is that oxandrolone as used did not decrease ultimate height, which is consistent with previous reports. The data strongly suggest that there may be an increase of 3 to 4 cm in the final adult stature of girls receiving treatment initially at 10 to 12 years of age. The mild androgenicity reported at a dose of .125 mg/kg/d has been observed by others, but seldom occurs if the dose is kept to <0.1 mg/kg/d. Further, these androgenic effects usually resolve when treatment is discontinued.*

Robert M. Blizzard, MD

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Mutation in the Gene Encoding the Stimulatory G Protein of Adenylate Cyclase in Albright's Hereditary Osteodystrophy (Pseudohypoparathyroidism)

G proteins are a large family of guanine nucleotide-binding proteins that facilitate signal transduction across cell membranes. Of the 3 subunits (α,β,λ) that comprise each G protein, the α subunit is thought to confer functional specificity to the molecule. One such protein, $G_S\alpha$, has been found to be defective in most cases of Albright's hereditary osteodystrophy (pseudohypoparathyroidism type Ia). $G_S\alpha$ is responsible for stimulating the hormone-sensitive adenylate cyclase system that generates the intracellular second messenger cyclic adenosine monophosphate (AMP). Albright's hereditary osteodystrophy (AHO) is an autosomal dominant disorder characterized by resistance of target organs to parathyroid hormone (PTH) and other hormones that utilize this signal transduction pathway.

Patten et al studied a family with AHO. They first showed that erythrocyte membranes from the affected mother and son (but not from an unaffected son) contained reduced $G_S\alpha$ bioactivity. They next demonstrated 2 populations of $G_S\alpha$ protein in the patients: 1 of normal and 1 of abnormal size. Analysis of genomic DNA subsequently showed the heterozygous loss of a restriction enzyme cleavage site in the 5' part of the $G_S\alpha$ gene; and sequencing of the region revealed a point mutation (ATG-to-GTG) in the initiator codon of 1 of the 2 alleles. The mutation was not present in normal individuals, nor was it detected in 7 other patients with $G_S\alpha$ deficiency.

An interesting aspect of this investigation was that although both the mother and son carried the same mutation, the son presented with the classic clinical and biochemical features of pseudohypoparathyroidism, including elevated levels of PTH. His mother had normal levels of calcium, phosphorus, and PTH and a normal urinary cyclic AMP response to bovine PTH. The authors speculated about why this should be but were unable to offer any firm explanations.

Patten JL, Hohns DR, Valle D, et al. *N Engl J Med* 1990;322: 1412-1419.

Editor's comment: *Although G protein abnormalities have been recognized for some time in this disorder, this is the first mutation of the $G_S\alpha$ gene to be reported. However, in an accompanying editorial, Spiegel (N Engl J Med 1990;322: 1461-1462) noted that 3 other mutations have been identified but not published. Thus, Albright's hereditary osteodystrophy is clearly a genetically heterogeneous disorder at the molecular level. Spiegel also addressed the variable expression issue, concluding with the suggestion that a 50% $G_S\alpha$ deficiency is necessary but insufficient by itself to produce full expression of the disease phenotype.*

William A. Horton, MD

In Future Issues

Obesity in Childhood and Adolescence, Part II: Pathophysiology, Association, and Complications
by W.H. Dietz, MD

Update: The Genetics of Insulin-Dependent Diabetes
by W.E. Winter, MD, and M.K. McLaren, MD

Support Groups for Individuals with Growth Problems and Their Families
by J. Weiss, MSW, LCSW, and J.G. Hall, MD

Abstracted from the work of:
Joseph D. Schulman, MD
Preimplantation Genetics
and
David L. Rimoin, MD
Limb Lengthening: Past, Present, and Future

Marfan Syndrome: The Basic Defect May Be in Sight

Marfan syndrome is the prototypical inherited disorder of connective tissue. Its manifestations, which involve the ocular, cardiovascular, and skeletal systems in particular, are familiar to most clinicians. Despite intense interest over the last several decades, its etiology and pathogenesis have remained elusive; however, 1990 has seen remarkable progress.

Godfrey and colleagues[1] demonstrated an apparent deficiency of elastin-associated microfibrils in skin and fibroblast cultures from affected members of 9 families. These fibrils constitute a fibrillar system that is widely distributed throughout the body, including tissues affected in Marfan syndrome. The fibrils are thought to serve as scaffolding for the deposition of elastin during elastogenesis. The samples were examined by immunofluorescence using monoclonal antibodies to fibrillin, a major structural protein of the microfibrils. The analysis was done "blindly," and deficient immunostaining cosegregated with the disorder when specimens from affected family members were compared with those from unaffected members, which stained normally. In an accompanying article, this group found similar microfibrillar abnormalities in skin biopsies and fibroblast cultures from the affected side but not from the unaffected side in a patient with asymmetric involvement of the syndrome.[2]

Hollister et al[3] next assessed fibrillin immunostaining in another group of patients with Marfan syndrome, as well as in patients with other connective tissue disorders. They confirmed the staining abnormalities, which included decreased numbers of fibers and abnormal staining patterns, in skin biopsies and fibroblast cultures from most Marfan patients (16 of 23 and 16 of 18, respectively). Seven of 25 patients with non-Marfan connective tissue disorders also showed abnormalities. These disorders included pseudoxanthoma elasticum, homocystinuria, ectodermal dysplasia, coronary artery dissection, cutis laxa, and epidermolysis bullosa-like syndrome. Interestingly, 3 Marfan patients had normal staining of skin, fibroblast cultures, or both.

Thus, microfibrils appear to be abnormal in most patients with Marfan syndrome. The abnormalities involve both the number and organization of the fibers, and are not completely restricted to Marfan syndrome. These observations led several groups to search for biochemical abnormalities of fibrillin in the syndrome. Indeed, McGookey et al[4] identified 3 types of abnormalities in fibrillin synthesis and secretion in fibroblasts from 21 patients. In 1 group of 7 probands, half the normal amount of fibrillin was synthesized, although it was secreted normally. Reduced synthesis and defective secretion were detected in the second group of 7 patients. Four patients in the third group of 7 patients showed normal synthesis and secretion, but defective incorporation of fibrillin molecules into the extracellular matrix. These types of abnormalities were consistent within families and were not found in unaffected members.

Coincident with these microscopic and biochemical studies have been attempts to map the gene locus of Marfan syndrome by reverse genetics. An international consortium was formed to pool linkage data from families with Marfan syndrome. Data from 25 three-generation families led to an exclusion map in which three fourths of the genome was excluded.[5] Searching for linkage in nonexcluded areas subsequently led Kainulainen and coworkers[6] to the long arm of chromosome 15. They studied DNA polymorphisms in 8 three-generation Finnish families with multiple affected members using anonymous chromosome 15q gene probes. Positive line of descent (LOD) scores were determined in 5 families, yielding a total score of 3.92. Since a score of >3.00 is considered strong evidence for linkage, these data provisionally map the Marfan locus to chromosome 15q. Confirmatory studies are now underway.

Editor's comment: *These reports demonstrate that the elusive basic defect in Marfan syndrome is falling victim to medical progress much as the molecular defects in many other genetic diseases have in recent months, eg, cystic fibrosis and neurofibromatosis. Despite several loose ends, such as failing to detect microfibrillar abnormalities in all Marfan patients while detecting abnormalities in some patients with other conditions, fibrillin has been established as a strong candidate for the mutant protein in Marfan syndrome. If the fibrillin gene locus can be mapped to chromosome 15q where the syndrome maps, although much work will still remain, the elucidation of the defect may not be far behind.*

William A. Horton, MD

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abnormalities with the Marfan phenotype in families. *Am J Hum Genet* 1990;46:652-660.

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Insulin-like Growth Factor II in Antenatal Growth

While considerable circumstantial evidence suggests that insulin-like growth factor II (IGF-II) plays an important role in antenatal growth, there is little direct evidence for this. DeChiara, Efstratiadis, and Robertson have provided such evidence through studies of mice in which the IGF-II gene was disrupted. Their strategy was to substitute by homologous recombination a mutated IGF-II gene for the endogenous (normal, wild-type) gene in embryonic stem (ES) cells. Essentially, 2 mutations were introduced into the gene that allowed for selection of cells expressing the mutant gene and also abolished the function of the gene product. Injection of ES cells, selected for the inactivated gene, into mouse blastocysts produced chimeric mice, which could be distinguished because the coat color of the host mice differed from that of the mice from which donor ES cells were obtained. Mating of male chimeric mice with germ cells thought to be derived from the injected ES cells to normal female mice generated offspring that were heterozygous for the inactivated IGF-II gene.

Comparison of newborn mice carrying a single dose of the functional IGF-II gene to newborn control mice carrying 2 doses of the gene revealed that the former were much smaller. Their weight was about half that of controls. Their absolute postnatal growth rate was also less than that of controls; however, when plotted as a function of birth weight, it was the same. Although the mice carrying the inactivated gene were not extensively evaluated other than for growth, they appeared to be otherwise normal, and they were fertile.

One of the most interesting observations was that mRNA transcriptase levels from the functioning IGF-II allele were approximately 10-fold less in heterozygote embryos compared with control mice embryos. Much less of a reduction had been predicted based on gene dosage, which was half, and weight, which was also about half. IGF-II peptide levels were not measured in the embryos. The authors were unable to explain this discrepancy but pointed out that the situation may be complex and needs further study. Breeding experiments to produce mice homozygous for the inactivated IGF-II gene are in progress.

DeChiara TM, Efstratiadis A, Robertson EJ. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* 1990;345:78-80.

Editor's comment: *This study provides direct evidence for the growth-promoting effects of IGF-II during in utero growth. The "apparent" normality of the heterozygous mice in non-growth-related characteristics raises the possibility that IGF-II, at least in the usual amounts, may not be essential for organogenesis in the early embryo. Breeding of mice in which both IGF-II genes have been inactivated should shed light on this matter and provide further insight into the role of this peptide in prenatal and postnatal growth in general.*

These studies were performed with mice for a variety of reasons. However, since IGF-II and its mRNA are widely distributed in human fetal tissues, the results presumably apply to humans as well.

William A. Horton, MD

Special Announcement

The 8th International Congress of Human Genetics, sponsored by the American Society of Human Genetics, will be held October 6-11, 1991 at the Washington Convention Center in Washington, DC. The deadline for receipt of abstracts is April 1, 1991. For abstract and registration forms or additional information, contact: M. Ryan, Meetings Manager, ICHG, 9650 Rockville Pike, Bethesda, MD 20814 USA (Telephone 301-571-1825; Fax 301-530-7079).

Treatment of Constitutional Delay of Growth and Puberty With Oxandrolone Compared With Growth Hormone

Twenty-six boys (12.1 to 15.9 years of age) with constitutional delay of growth and puberty (CDGP) were given either 20 U/m²/wk (approximately 0.3 mg/kg/body weight) of growth hormone (GH) or 2.5 mg of oxandrolone per day. The former group received GH for 12 months and the latter received oxandrolone for the first 3 of 12 months. At the end of 12 months, the boys in both groups grew significantly and advanced toward puberty at comparable rates. These data are presented in the table.

Mean	Growth Hormone		Oxandrolone	
	Before Rx	After Rx	Before Rx	After Rx
Chronologic age (yr)	13.9	15.0	13.8	14.8
Testicular volume (cc)	4.8	10.7	5.8	10.0
Growth velocity (cm/yr)	3.8	6.8	3.9	8.3
Bone age delay (yr)	-2.3	-3.0	-2.1	-2.2
Standard deviation score for bone age	-0.6	-0.1	-0.8	-0.2

Bone age advancement was comparable, as was the increase in testicular volume. Only 3 of the oxandrolone-treated group and 2 of the GH-treated group had testes greater than 15 cc at the end of the treatment year.

The authors conclude that GH is not indicated for the management of delay in the pubertal growth spurt. Oxandrolone can be very beneficial.

Buyukgebiz A, Hindmarsh PC, Brook CGD. *Arch Dis Child* 1990;65:448.

***Editor's comment**: Oxandrolone is used by both the British and US endocrinologists for treatment of CDGP but is used differently. The British induce puberty over a 3-month period in boys who have testicular volume of at least 4 cc. The Americans provide the drug over an extended period until the patient is secreting significant testosterone (>100 ng/mL). Possibly the Americans may wish to evaluate the length of treatment after considering the data of Buykgebiz et al.*

Robert M. Blizzard, MD

Final Height in Boys With Untreated Constitutional Delay in Growth and Puberty

Short stature due to constitutional delay in growth and puberty (CDGP) is the most frequent cause of short stature referred to the pediatric endocrinologist. Although an extreme of normal development rather than a clinical disorder, it can still pose clinical concerns for patients, parents, and physicians. These patients are believed to grow to a normal height and, therefore, treatment with growth-promoting agents is primarily for psychological reasons. Crowne et al undertook a retrospective study (1976-1986) of patients with CDGP to determine the natural history of growth patterns and psychological impact of these growth patterns in 118 boys with CDGP. Forty-three were followed to final height.

At presentation, the mean chronologic age (CA) was 14.0 years ±1.9 SD, the mean bone age (BA) delay, 2.7 ± 1.0 years, and height SD score (SDS), -3.4 ± 0.6. The predicted adult height SDs by the Tanner-Whitehouse II method was -1.3 ± 0.7 years. The "final" adult height SDS measured when all patients were more than 21 years of age or growing less than 2 cm per year was reported at -1.6 ± 0.9 SD.

Comparison of final adult height and midparental height revealed a significant difference of -6.5 ± 6.0 cm. In 14 of the 20 sets of parents who were measured and who reported their heights, the measured heights were less than 1 cm different than those reported. In 6 sets of parents, the difference was greater than 2.5 cm, with the reported height being greater. Regardless, when the measured height was used, the difference between midparental height and final height was significant (P = 0.003).

In respect to self esteem, *there was no significant* difference between the boys with CDGP and a normal control group, as measured by the Coopersmith self-esteem inventory. Further, there was no significant difference between the groups in recorded social activity, number either married or in

stable relationships, or in the number of unemployed. Less than 10% think of their height currently or on an occasional basis only.

Seventy-nine percent of the CDGP group are satisfied with their height versus 99% of the control group. Fifty percent would have liked to have had treatment to bring on their growth spurt, and 55% would access treatment for their children should they be faced with a similar growth problem.

Crowne EC, Shalet SM, Wallace WHB, et al. *Arch Dis Child* 1990;65:1109-1112.

Editor's comment: *The authors conclude that the results concerning the difference in midparental heights and final predicted heights deserve further study to determine if boys with CDGP are shorter than expected for their midparental heights and to determine whether growth-promoting agents enhance the heights of boys with CDGP. They also conclude that the principal reason for treating boys with CDGP with growth-promoting agents is to alleviate short-term stress and distress. The fact that 55% of those responding wanted their children treated in order to avoid the stresses they had endured as adolescents justifies treatment consideration in certain patients with CDGP.*

Robert M. Blizzard, MD

Growth of Males With Idiopathic Hypopituitarism Without Growth Hormone Treatment

Twenty-three males with idiopathic hypopituitarism who were not treated with growth hormone (GH) were evaluated with respect to their ultimate adult height. A majority of these individuals were born by breech delivery, which accounted for the hypopituitarism. A majority also had gonadotropin deficiency in addition to GH deficiency. Treatment with androgen in those with gonadotropin deficiency was started at a mean age of 17.4 years. At that time, all patients had heights -3 SDs below the mean. Bone maturation was greatly retarded, with bone ages (BAs) more than 3 years below the chronologic age (CAs). Patients whose puberty developed spontaneously had comparable BAs and heights when puberty began.

Patients with "induced" puberty reached a mean final height of 157.0 cm at a mean age of 26.1 years. The mean adult height was -3.9 SD for 4 patients with spontaneous puberty and -3.1 SD for the 19 with "induced" puberty. The pubertal period had a mean duration of 8.7 years in these 19 patients, during which height increased by a mean of 20.4 cm. The mean difference between the predicted adult height at the onset of "induced" pubertal growth and the attained final height was -7.1 cm, ranging from -24 cm to +4 cm. Adult heights were positively correlated with heights at the onset of pubertal growth. The total mean height gained during "induced" puberty (20.4 cm) compares favorably with the height gained during spontaneous puberty by normal late maturing boys (approximately 18 cm).

The authors conclude that physicians should make every effort possible to increase the heights of GH deficient patients to within the normal range before puberty begins. If this is not done, significant short stature will persist in adulthood.

Van der Werff ten Bosch JJ, Bot A. *Clin Endocrinol* 1990; 32:707-717.

Editor's comment: *The authors document the ultimate heights of patients with hypopituitarism seen in their clinic. There are few tabulations of final adult heights of hypopituitary patients not treated with growth hormone, and this paper is a significant contribution in this respect. It is surprising that the mean growth attained following initiation of testosterone treatment was 20.4 cm. This was over a protracted treatment period, and probably results from relatively low doses of replacement therapy. It is to be noted that the vast majority of the patients did not reach their predicted heights.*

The role of GH is important in the treatment of the GH deficient patient. Early diagnosis and adequate treatment are necessary for individuals with GH deficiency to reach their predicted heights or a height in accord with the target range based on midparental height. These are the emphatic points made by the authors and this editor.

Robert M. Blizzard, MD

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Heritable Origins of Type I (Insulin-Dependent) Diabetes Mellitus: Immunogenetic Update

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Introduction
The etiology of type I (insulin-dependent) diabetes mellitus (IDDM) is multifactorial. Studies of identical twins reveal maximum concordance rates of 50%, suggesting that environment, in addition to heredity, influences the development of pancreatic beta-cell autoimmunity. Susceptibility to IDDM is not inherited as a simple mendelian trait since only 5% to 10% of patients with IDDM have a parent or other first-degree relative affected with IDDM.

Genetic predisposition to IDDM is most strongly associated with specific HLA-DR and HLA-DQ alleles of the major histocompatibility complex (MHC) located on the short arm of chromosome 6. Other candidate genetic loci—including the insulin gene (chromosome 11), κ light-chain gene (chromosome 6), immunoglobulin heavy-chain gene (chromosome 12), and Kidd blood group—have little or no influence on inherited susceptibility to IDDM. Although unmapped, autosomal dominant thyrogastric autoimmunity provides increased proclivity to IDDM. Gender has a modest influence on predisposition to IDDM early in life as males are in definite excess by ~20% in IDDM onset at 5 years or less. By adolescence, males and females are affected equally frequently.

Class II MHC molecules, antigen-derived peptide fragments, and T-cell receptors are necessary for the generation of immune responses. Once nominal pancreatic beta-cell antigen is available, variations in antigen structure between controls and IDDM patients can be sought. The present search for genetic susceptibility to IDDM has included studies of HLA class II and T-cell receptor genes.

Letter From the Editor

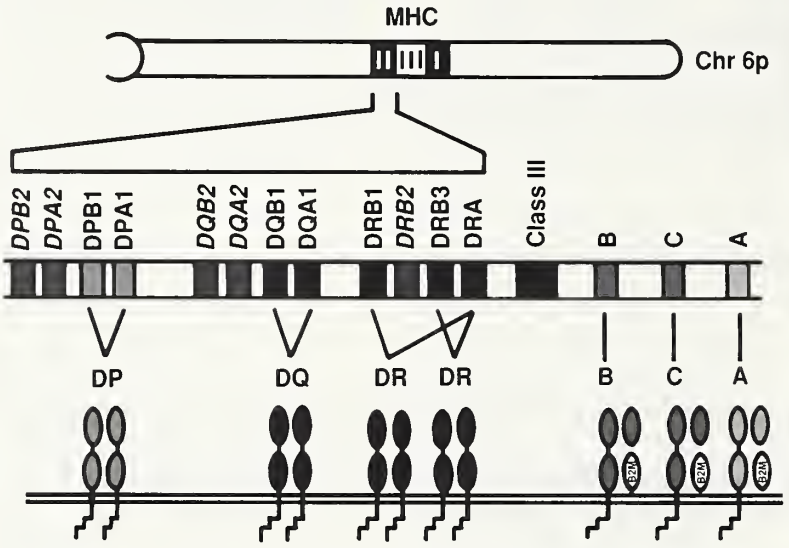
Although a section (Letters to the Editor) has been dedicated to receiving comments, few have been forthcoming the past 2 years. You as a colleague and reader are encouraged to challenge our editors, bring new information to the attention of our readers and ourselves, and probe for different or additional information. This section is too important for it to lapse because of disuse. Please let us hear from you.

From the Editorial Board
Robert M. Blizzard, MD

In This Issue

Assisted Reproductive Technologies and Preimplantation Genetics – see page 6

Figure 1



The human MHC is located on the short arm of chromosome 6 (Chr 6p). Class I MHC genes code for a 44-kD glycoprotein present on all nucleated cells that noncovalently associates with the non-MHC protein β2M (open ellipses). Class II MHC molecules, which are normally restricted to antigen-presenting cells and B lymphocytes, are coded for by separate α and β genes coding, respectively, for 34-kD α chains and 29-kD β chains. The class III MHC region includes loci for the adrenal cortical enzyme 21-hydroxylase gene, the complement protein C4 gene, TNF-α gene, and TNF-β gene. In this diagram, the class II MHC genes are named using the 1989 histocompatibility workshop nomenclature.

Table 1
Class II MHC Gene Terminology
(Nonexpressed genes are in italics.)

Nomenclature	
Pre-1989	1989
DPβ2	*DPB2*
DPα2	*DPA2*
DPβ1	DPB1
DPα1	DPA1
DXβ	*DQB2*
DXα	*DQA2*
DQβ	DQB1
DQα	DQA1
DRβI	DRB1
DRβII	*DRB2*
*DRβ*III	DRB3
DRα	DRA

An illustrated key to the new taxonomy. The DRA chain can pair with either of 2 DRB chains to produce 2 possible DR molecules. The DQA1 and DQB1 chains pair to form the DQ molecule, while DPA1 and DPB1 chains pair making the DP molecule. *DRB2*, *DQA2, DQB2, DPA2*, and *DPB2* are pseudogenes and as such are not expressed.

MHC Genes in IDDM: Studies in Humans

The MHC (Figure 1 and Table 1) controls which antigens an individual responds to and the degree of the response. Class I molecules are glycoproteins coded for by single genes within the MHC (HLA-A, HLA-B, and HLA-C) located on the exterior of all nucleated cells. They survey the intracellular milieu in order to present endogenous or invading antigens as possible targets of CD8 cytotoxic lymphocytes. Class II molecules (HLA-DR, -DQ, and -DP) are heterodimeric cell surface glycoproteins composed of an α and a β chain coded for by 2 separate genes within the MHC. Macrophages digest phagocytized matter to release antigen-derived peptides. These fragments, which are initially of extracellular origin, bind to class II MHC molecules to be presented to CD4 T lymphocytes, which in turn orchestrate the immune response.

In the 1970s, increased frequencies of the class I MHC alleles HLA-B8 and -B15 were noted in patients with IDDM compared with controls. With the developments of Dw (primed lymphocyte) cellular typing and HLA-DR serotyping somewhat later, IDDM was shown to be more closely associated with certain class II MHC alleles than class I MHC alleles. Empiric risks for IDDM based on HLA-DR phenotype have been previously reviewed in *GGH,* Volume 2,

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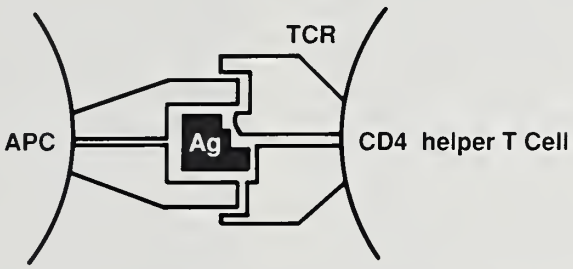
Figure 2



Class II MHC molecules of antigen-presenting cells (APC) display antigen-derived peptide fragments (Ag) to the T-cell receptors (TCR) of CD4 helper T cells. APCs such as macrophages consume cellular debris and exogenous antigens, and degrade the ingested proteins into peptides. These peptides in turn are displayed on the APC cell surface cradled in the peptide binding cleft or groove of the class II MHC molecule. Theoretically, if a TCR recognizes the MHC beta-cell autoantigen peptide assembly, an immune response against the pancreatic beta cell is initiated and the eventual clinical consequences are insulinopenia and IDDM.

No. 1 (1986). Serologic studies in whites demonstrated that HLA-DR3, -DR4, and -DR1 were positively associated with IDDM while HLA-DR2 and -DR5 were negatively associated with IDDM. The other DR serotypes have a neutral effect on predilection to IDDM. Of interest, HLA-DR1 was a risk allele only when associated with DR3 or DR4, while the protective effect of DR2 was greater than that of DR5. Blacks exhibited a consistent association of HLA-DR4 and a weaker relationship of DR3 with IDDM. In contrast, Japanese IDDM patients displayed increased frequencies of HLA-DR4 and -DRw9.

Since no HLA-DR associations with IDDM proved to be absolute and since specific HLA-DQ and HLA-DR alleles are in tight linkage disequilibrium, recent investigations have examined the possible relationship of HLA-DQ to IDDM. Variability in HLA-DR alleles is strictly due to the genetic diversity of DRβI (DRB1) or *DR*βIII (DRB3) since DRα (DRA) is essentially nonpolymorphic. In contrast, diversity in DQα (DQA1) and DQβ (DQB1) chains can both contribute to DQ variability. Researchers have examined the *hypothesis* that serologically defined HLA-DR types might be further partitioned into diabetes-prone and diabetes-resistant alleles using DQα or DQβ typing. These studies were initiated in 1983 by David Owerbach, who used restriction fragment length polymorphism (RFLP) analysis of DR4-associated DQβ gene alleles. He found that a DQβ *Bam*HI 3.7-kb fragment was associated with resistance to IDDM. In addition to the DR4-DQβ subtypes, many studies followed that delineated RFLP subtypes of DQβ chains of DR2 and DR6 haplotypes that defined susceptibility or resistance to IDDM.

DXα (*DQA2*) and *DXβ* (*DQB2*) are located between DQ and DP. *DXα* has been implicated as a modifier of genetic susceptibility; however, a DX molecule is not expressed. Associations of *DXα* alleles and IDDM appear solely to reflect linkage disequilibrium of DX with DR and DQ alleles in extended HLA haplotypes that display resistance or proneness to IDDM. As well, HLA-DP (DPα [DPA1] and DPβ [DPB1] chains) does not appear to contribute to susceptibility to IDDM as DP and DQ are separated by α and β chains of the class II molecule (Figure 2). Each chain contributes an α helical "wall" and 4 β pleated sheets of the cleft "floor" to form a peptide binding pocket (2 α helical "walls" and a "floor" of 8 β pleated sheets). Polymorphic amino acids, which produce allelic variability, generally face into the cleft to influence peptide binding. DQβ β 1 exon amino acid 57 is located at the beginning of an α helix. Theoretically, negatively charged aspartic acid at this position can alter peptide binding by facing into the cleft, or by forming a salt bridge with a conserved DQα chain arginine at position 79. Also, as helical amino acids can interact with T-cell receptors, the strength of MHC-T-receptor interactions thus could be modified, further influencing the immune response.

Several evaluations of DQβ position 57 have been performed in various populations. When 39 IDDM patients were genotyped by Todd et al for the presence or absence of the putative critical amino acid, 90% were found to be non-aspartic acid homozygotes while only 10% were heterozygotes and *none* were aspartic acid homozygotes. The association of non-aspartic acid residues at DQβ position 57 with IDDM has been recognized in population studies from the United States, Denmark, Finland, Norway, and France. However, from these studies, HLA susceptibility to IDDM was not strictly recessive, since some individuals heterozygous at position 57 (aspartic acid/non-aspartic acid) or homozygous for aspartic acid had IDDM. Furthermore, if HLA susceptibility was strictly recessive, 100% of affected sib pairs in multiplex families would be HLA identical. This is not the case, as only 60% of cases are HLA identical while 35% are haploidentical (ie, share 1 HLA haplotype) and a minority (≤ 5%) of sibships are HLA

nonidentical (ie, share no HLA halotypes).

From the above analysis it is also apparent that DQβ alleles containing aspartic acid at position 57 are not uniformly protective of the development of IDDM. The only allele to provide dominant protection is the DR2-linked DQβ1.2. Even in association with DR4-DQβ3.2, DR2-DQβ1.2 prevents IDDM. Rarely, DR2 alleles are seen in IDDM patients; however, in such cases, the DR2-linked DQβ (DQβ1.AZH) allele lacks aspartic acid, which is consistent with the association of IDDM and non-aspartic acid residues at DQβ position 57. While DR5, like DR4, can be linked to either DQβ3.2 or DQβ3.1, DR5 is most commonly linked to DQβ3.1, a nonsusceptibility allele, which explains the protective effects of most DR5 alleles.

Non-aspartic acid 57 alleles do not provide equal disease susceptibility (strength of susceptibility: DQβ3.2 > DQβ2 > DQβ1.1 > DRβ1.AZH), nor are all aspartic acid 57 alleles equally neutral or protective (strength of protection: DQβ1.2 > DQβ3.1). In one study, DQβ3.2 was more strongly associated with IDDM than DQβ2. This implies that amino acids other than those at DQβ position 57 modify susceptibility or resistance. DRβI and DQα genes may also temper DQβ genetic susceptibility to IDDM. For example, Sheehy et al simultaneously studied DQβ alleles and DR4-DRβI alleles (Dw4,Dw10, etc) to demonstrate their interaction. DR4-Dw4,DQβ3.2 or DR4-Dw10,DQβ3.2 displayed a relative risk for IDDM of 38, which was several times higher than risk assessments based on DR or DQβ allele typing alone.

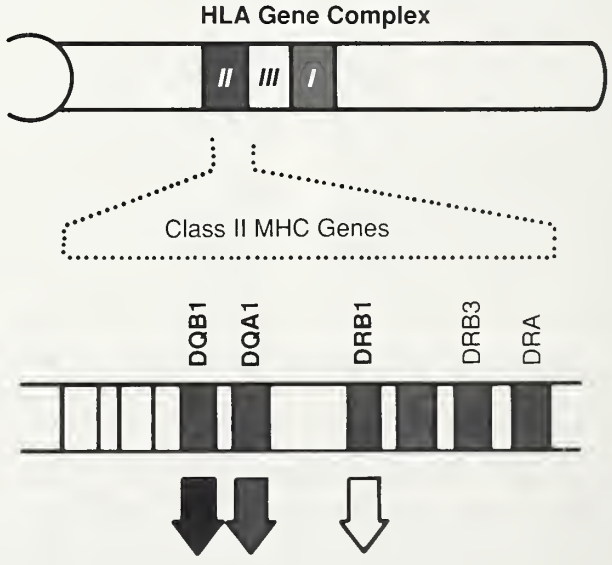
In some researchers' hands, DQβ assessment has increased the predictability of IDDM. In a US population, Morel et al reported that individuals homozygous for non-aspartic acid 57 DQβ alleles had a relative risk for IDDM of 107. In France, individuals heterozygous for DQw2,DQw3.2 (both alleles lack aspartic acid at position 57) had a relative risk for IDDM of 52.9. Such estimates, approaching 1 in 5, compare favorably with empiric IDDM risks in identical twins (~1 in 3 to ~1 in 2). In siblings who are HLA identical to a diabetic proband, the absolute risk for IDDM is ~1 in 7 and rises to ~1 in 4 if HLA-DR3 and -DR4 are shared. However, in the French studies, non-aspartic acid 57 homozygosity alone was less predictive of IDDM, with a relative risk of 13.2, comparable with that of the 17.5 in Finns and 12.2 among Norwegians. Recently published risk estimates from Texas are even more conservative, with a relative risk for IDDM of 4.5 in aspartic acid-negative homozygotes.

Important exceptions have arisen to the correlation of diabetes susceptibility with specific charged or neutral amino acids at DQβ position 57. As noted above, it has become apparent that a small percentage of IDDM patients are, in fact, homozygous for aspartic acid-positive DQβ alleles, and that in DR1,DR4 heterozygosity, DQβ3.2 is not increased in frequency. Thus, in DR1,DR4 heterozygous IDDM patients, DR1 (DQβ1.1, aspartic acid negative) appears to supply the diabetes predilection.

A particular problem for the DQβ position 57 theory is DR7. Although DR7 is linked to DQβ2, as is DR3, DR7 is not associated with IDDM in whites. One possibility to consider in this regard was the influence of the respective DQα alleles, as the DQ molecule is a heterodimer. While studies on the influence of the DQα to date have been limited, Todd and colleagues have pointed out that the DR7 in whites is linked to the DQα A2 allele, in

Figure 3

Genetic Susceptibility To Type I Diabetes (IDDM)



Alleles at the DQB1, DQA1, and DRB1 loci influence susceptibility to type I diabetes (IDDM). The darker the arrow, the relatively greater influence the locus has on predilection to IDDM. Only the expressed loci are labeled and the DP loci are not depicted (see Figure 1 for comparison).

contrast to the DR3 in whites which is linked to the DQα A3 allele. In blacks, however, DR7 can be linked to DQα A3 and DQβ2, similar to white DR3, and thus can be associated with IDDM like DR3 in whites. Thus, the DQα A3 allele may be required in addition to DQβ2 to confer diabetogenicity (Figure 3, page 4).

Another challenge to the DQβ position 57 theory comes from studies of IDDM patients in Japan. Japanese patients with IDDM do not demonstrate an increased frequency of aspartic acid-negative DQβ alleles. Whereas the low number of non-aspartic acid alleles is consistent with the low prevalence of IDDM in the Japanese (~1:10,000 vs ~1:500 for the US), those with IDDM may carry a DQα A3 susceptibility allele to account for HLA susceptibility in Japan. Of interest, in black patients with IDDM from Zimbabwe, only DQα RFLPs correlated with susceptibility to IDDM. The weak relationship of DR3 to IDDM in blacks may reflect the linkage of DQw4 (IDDM nonassociated) to DR3 in this ethnic group. In whites, DR3 is exclusively linked to DQβ2.

Aspartic acid position 57-diabetes susceptibility correlations also do not explain why DR3,DR4 heterozygosity produces the highest relative risk for IDDM. Nepom et al demonstrated that the DR3-linked DQw2 α chain can pair with the DR4-linked DQw3 β chain, producing a novel class II MHC product. However, such transcomplementation occurs in controls as well as in patients with IDDM and thus is not unique to patients with IDDM.

MHC Genes in IDDM: Studies in Nonobese Diabetic Mice

The nonobese diabetic (NOD) mouse experiences spontaneous autoimmune destruction of pancreatic beta cells and is thus an excellent model for human IDDM. As in humans, IDDM in NOD mice is multifactorial, and up to 6 autosomal recessive genes are postulated. According to RFLP studies and DNA sequencing, the NOD class II molecule IA (homologous to DQ) is unique. Similar to several other strains of mice, NOD mice do not express a class II IE molecule (homologous to DR). Predilection to insulitis and IDDM is strongly associated with IA^{nod}. In outbreeding experiments, diabetic mice are homozygous for IA^{nod} with only rare exceptions.

Similar to DQβ susceptibility alleles in humans, $A\beta^{nod}$ at the β 1 domain position 57 lacks aspartic acid, as $A\beta^{nod}$ is positive for serine. However, we now recognize that other strains of mice also lack aspartic acid at Aβ position 57 and do not develop IDDM. Boehme and coworkers have identified at least 7 Aβ alleles in various *Mus* species (my1, Eccles, W250, STC90, W253, stf, and zbn) that carry a neutral amino acid at Aβ position 57.

Recent experiments in transgenic NOD mice have directly addressed the issue of aspartic acid 57 in $A\beta^{nod}$. When NOD mice were made transgenic for IA^{K} (aspartic acid 57 positive), insulitis was prevented. This is consistent with the hypothesis that the Aβ gene on chromosome 17 is the locus of diabetes susceptibility or resistance. Other investigators mutated the transgenic $A\beta^{K}$ position 57 to serine but did not restore diabetogenicity to the allele. Furthermore, in a third study, amino acid 56 of $A\beta^{nod}$ was mutated from histidine to proline, preventing diabetes in the model.

These studies demonstrate that position 57 of $A\beta^{nod}$ is not exclusively responsible for susceptibility or resistance and other class II MHC amino acid motifs may impact extensively on diabetogenicity. The transgenic expression of IE, either through breeding or direct microinjection, is also able to prevent pancreatic beta-cell autoimmunity in NOD mice. Thus, 2 elements of the NOD MHC are required for diabetogenicity: a novel IA molecule and the absence of IE expression.

T-Cell Receptor Genes in IDDM: Studies in Humans and NOD Mice

Concurrent with the above analyses, α/β T-cell receptor inheritance has been studied. While there was no consensus of opinion among early investigations concerning T-cell receptor β inheritance and IDDM, Concannon et al recently demonstrated in human sib pair analysis that T-cell receptor β polymorphisms were not associated with IDDM. The data are consistent with our own published work in the NOD mouse in which we were able to demonstrate that T-cell receptor β^{nod} did not function as an autosomal recessive autoimmunity gene in influencing the development of insulitis in ([NOD x NZW]F1 x NOD) backcross mice. In a limited population of such backcross mice, we also demonstrated that neither T-cell receptor α^{nod} nor γ^{nod} modified genetic susceptibility to pancreatic beta-cell autoimmunity, excluding a genetic influence derived from genes coding for γ/δ T-cell receptors. A similar conclusion for T-cell receptor α was drawn in NOD, NON outcross, backcross experiments. Unresolved is the question of whether specific Vβ or Vα segments are utilized in the immune response to pancreatic beta-cell autoantigen. This issue is a postfertilization concern and does not relate to inherited proclivity for IDDM.

Chromosome 9 Genes in IDDM: Studies in NOD Mice

Studies in a NOD x NON outcross-NOD backcross by Prochazka and colleagues and by Hattori and colleagues suggested that murine chromosome 9 influenced predilection to IDDM. Initial findings indicated that the chromosome 9 IDDM susceptibility gene mapped centromeric to Thy-1. However, markers centromeric of Thy-1 showed weaker associations with IDDM than Thy-1. Thus, any pancreatic beta-cell autoimmunity gene on chromosome 9 would not function as an absolute

autosomal recessive. Chromosome 11 in humans, which is homologous to murine chromosome 9, does not appear to be associated with IDDM.

Summary

As human IDDM is clearly polygenic in etiology, future genetic analysis might best be undertaken in the NOD mouse model. When regions of the murine genome show potential association with pancreatic beta-cell autoimmunity, the studies can then be undertaken in humans. Reverse genetics can be employed using random, regularly spaced DNA probes in NOD outcrosses to identify previously unknown immunoregulatory genes of importance to the inheritance of IDDM. The creation of transgenic mice will allow the direct testing of molecular theories of IDDM in vivo. In the near future, we expect to be able to predict genetic susceptibility to IDDM with considerable certainty. In humans, future studies should simultaneously assess DQβ, DQα, and DRβ gene alleles in large numbers of subjects of diverse ethnic origin to further unravel the class II MHC relationships with IDDM. The long-term goals of those studies would be to develop accurate estimates of susceptibility based on the entire HLA haplotype of an individual.

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Additional references available upon request.

Assisted Reproductive Technologies and Preimplantation Genetics

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Introduction

In his presentation of this topic at the Genentech National Cooperative Growth Study held in Fort Lauderdale, Florida, on November 10 - 13, 1990, Dr. Schulman discussed techniques of in vitro fertilization, the technology of preimplantation genetics, and current ethical considerations for embryo implantation. He completed his discussion with some considerations for future research. The presentation was exceedingly informative and lucid. To the extent possible in a short abstract of the presentation, the Editorial Board wishes to share this information with the readers.

In Vitro Fertilization

Historically, the first birth from in vitro fertilization (IVF), the famous Louise Brown, occurred in 1978 in England as a result of the efforts of Drs. Patrick Steptoe and Robert Edwards. Today, over 5,000 births have occurred as a result of in vitro fertilization. The incidence of malformations has been similar to those observed in infants conceived in vivo and born to similar maternal age groups. The common indications for doing IVF are given in Table 1, page 7.

The methodology is geared to obtain multiple embryos in

Table 1
Indications For In Vitro Fertilization

Tubal Factors	Tubal obstruction (when surgery has failed)
Peritoneal Factors	Peritonitis encasing ovaries or reproductive organ with distortion of ovary and fallopian tubes
Male Infertility	Mild to moderate sperm defect that requires treatment or separation of sperm
Endometriosis	Patients failing to respond to usual surgical or pharmacologic treatment
Ovarian Failure	Premature menopause; requires ovum donor
Immunologic Factors	Patients with antisperm antibodies; requires special handling of sperm

order to maximize success. Therefore, Pergonal®, a gonadotropin mixture from the urine of postmenopausal women, is frequently used to induce maturation and ovulation of multiple oocytes. The retrieval of ova by laparoscopy has been replaced by transvaginal ultrasound techniques. The advantages of this approach are its speed, reduced risks to the patient, lower costs, the ability to circumvent extensive pelvic disease, and the use of local rather than general anesthesia.

A needle, pushed about 1 to 2 cm through the top of the vagina, can readily enter the ovary, and ova are then aspirated. The process takes about 15 minutes and if there has been a good response to gonadotropins, an average of 5 to 7 eggs can be recovered. The eggs are transferred into special culture dishes containing a solution, highly controlled for pH ion concentration, that permits effective production of embryos from insemination with a tiny amount of washed sperm.

Sixteen or 18 hours post-fertilization, the male and female pronuclei become clearly visible within the zygote, providing conclusive evidence that fertilization has occurred. The zygote undergoes cleavage and by 40 hours embryos of 2 cells are formed. These are then transferred into the uterus after 2 to 3 days when the morula reaches the 2- to 8-cell stage. More fully developed blastocytes can also be transferred.

A large proportion of the IVF pregnancies (~50%) in the United States occur in the 9 or 10 largest centers where 500 to 700 IVF cycles per year are performed. Unfortunately, only a small number of centers offer high-quality embryo freezing programs. This facilitates the establishment of pregnancy safely when many embryos are produced, and also can facilitate the diagnosis of normalcy of embryos subjected to preimplantation genetic testing.

Major determinants of IVF success include the woman's age; the response to ovarian stimulation, which depends upon the oocyte number and quality; the sperm quality; the fertilization rate or embryo number; and appropriate hormone levels. Maternal age has a marked effect on the efficiency of assisted reproductive technologies. A woman 25 years of age has a 25% chance of pregnancy with each IVF cycle. The woman who is 42 years old will have about a 5% pregnancy rate. In the younger age group, 20% to 25% become pregnant with a simple embryo transfer, 30% to 40% with 2 transfers, 45% or more with 3, etc. There is also a significant difference in the miscarriage rates depending on age. The IVF miscarriage rate is probably similar to that in natural pregnancies. In women in their 20s, the miscarriage rate is about 10%; for those in their 30s, about 20% to 25%, and about 35% to 40% in women in their 40s. A woman in her 20s will have approximately a 23% live birth rate, while a woman in her 40s will have about a 3% rate.

Implantation Genetics
Genetic considerations for the implanted embryo are the same as those in most pregnancies. However, amniocentesis, chorionic villus sampling (CVS), and intravaginal ultrasound studies are more frequently performed.

Preimplantation Genetics
Embryo biopsy before implantation and analysis of oocytes and sperm before conception are the most recent developments in this rapidly advancing field. The major X-linked defects may be approached utilizing DNA-based techniques. A not remarkable scenario is one in which the embryos are sexed, and only female embryos are replaced into the uterus. This avoids the birth of a child with X-linked diseases such as Duchenne type muscular dystrophy, hemophilia, fragile X syndrome, etc.

Preimplantation testing is an exciting alternative to CVS. The early morula is biopsied, usually at the 8- to 16-cell stage. If necessary, the embryo is frozen to permit time for the genetic analysis. DNA from a single cell or a pair of cells is amplified utilizing PCR. However, this analysis is difficult, and it is uncertain whether or not single copy genes can be reliably amplified starting with 1 or 2 molecules of DNA. It is very important in these studies to avoid sperm contamination of the biopsied

material. Since 100,000 sperm are in the dish with 1 egg, this is difficult. Sperm DNA is extremely difficult to amplify because it is highly concentrated unless it is treated with dithiotheritol or certain other agents. It is recommended that female laboratory technicians preferentially perform these studies in order to reduce the risk of false results caused by contamination of Y chromosome material from male skin cells.

Fortunately, preimplantation testing is not limited to PCR-based techniques. It soon may be possible to perform biochemical analysis on individual blastomeres such as has been done in mouse embryos. It also may be possible to look at the possibilities for in situ hybridization or actual karyotyping of single blastomeres from embryos. In performing 1-cell biopsies, the embryo is held against the tip of a pipette, and a fine glass needle or acid Tyrode's solution is then used to make a defect in the zona pellucida, and a blastomere is removed by gentle suction.

Table 2
Inherited Defects

Dominant Inherited Conditions
- Huntington's disease
- Neurofibromatosis
- Marfan syndrome
- Achondroplasia
- Charcot-Marie-Tooth disease
- Familial hyperthermia
- Numerous sublethal diseases

Resessive Inherited Conditions
- Cystic fibrosis
- Sickle cell anemia
- Thalassemias (α,β)
- Tay-Sachs disease
- Gaucher's disease
- Familial spinal muscular atrophy (Werdnig-Hoffman paralysis)
- α_1 antitrypsin deficiency
- Numerous other lethal conditions

Currently, reported pregnancies following preimplantation testing are limited to the Hammersmith Hospital team in London. They have produced 5 or 6 pregnancies by this method. In all cases, these were families with a high risk for X-linked disorders of various types. The investigators did not do specific testing for Duchenne type muscular dystrophy or other diseases, but biopsied the embryos in the 6- to 8-cell stages and then did PCR analysis using probes for Y-specific repeat sequences. These studies are still very investigational.

The diseases that, hopefully, can be prevented by preimplantation genetics and that are currently under investigation are listed in Table 2. As mentioned previously, it may be possible to do enzyme assays on 1 or 2 cells from embryos. This has been possible in a mouse test system where the enzyme associated with Lesch-Nyhan syndrome has been successfully measured in individual blastomeres. It is important to be certain that one is measuring a true embryo enzyme level, since the oocyte has its own cytoplasmic enzyme. The first few divisions of a human embryo are reduction divisions and, unfortunately, new enzyme synthesis may not occur until about the 8-cell stage. This is in contrast to the mouse, where the embryo begins making its own enzyme around the 2-cell stage. Consequently, there is concern that biopsying and measuring enzyme levels in 6- or 8-cell human embryos may give incorrect conclusions about whether or not one has an affected embryo. However, it is certainly possible to do enzyme studies on later embryonic stages (late morulas or blastocysts), and this is something that will probably occur in the future. Embryos at the late morula or early blastocyst stage can be replaced into the uterus and a certain number of pregnancies will occur.

In situ hybridization involves using chromosome-specific probes that are usually fluorescent labeled, will hybridize on a slide to metaphase preparations, and can even be used to examine interphase nuclei. Bright spots occur, which presumably are representative of the region of the chromosome to which they are bound. A number of chromosome-specific probes are available now for in situ study. Such studies should open the door to embryonic chromosome analysis in certain high-risk situations.

Uterine lavage is another technique that has potential to alleviate some of the problems of preimplantation manipulation. Following insemination, a special catheter is placed into the uterus through the cervix; the uterus is then lavaged and embryos are recovered. The risks involved are embryo retention and failure to recover the embryo. Embryo retention can be dealt with pharmacologically while failure of recovery can hopefully be addressed with improved catheters. In this technique, one looks for recovery of blastocysts because the embryo does not enter the uterine cavity until just before the blastocyst stage — at 16 to 32 cells. Since blastocysts have many cells, they are good candidates for preimplantation diagnosis. However, one might not always get the information reflective of the genotype of the embryo inside if one biopsies the trophoblastic outside of a blastocyst.

Separation of X-Bearing and Y-Bearing Sperm

The separation of X-bearing and Y-bearing sperm is potentially possible. Theoretically, this technique has the advantage of permitting insemination with only X-bearing sperm, thus eliminating X-linked diseases. Techniques have been developed that use albumin or other gradients to separate the lighter from heavier sperm (Y from X), but it does not appear to be reliable. Recently,

workers at the United States Department of Agriculture (USDA) in Beltsville, Maryland, have developed an approach where sperm are exposed to a DNA dye and then fluorescence-activated sorting is used. The instrument to separate the sperm can be adjusted in such a way that the Y sperm, which have about 3% less DNA than the X-bearing sperm, may be sorted with some degree of reliability. With as much as 5% or 6% difference in DNA content of the X and Y sperm, extremely reliable separation of the X and Y embryos has been done in nonhuman species. Studies are being conducted in collaboration with the investigators in Beltsville to see whether this technique can be accomplished with human sperm. Unfortunately, human sperm and those of some of the higher primates tend to be polymorphic, which makes the technique more difficult to implement. However, it should be possible in the future to accomplish this, and this will be a very powerful approach to eliminating X-linked disorders.

Preconception Analysis of Oocytes

Preconceptive testing of oocytes would obviously cause cell death. However, biopsy of the polar body, which is presumably the genetic mirror image of what is left in the egg, is feasible. For example, if the DNA of the polar body had the cystic fibrosis gene, then one could say by implication that the egg itself would not. Then the egg could be inseminated, and, theoretically, the embryos resulting would be free of cystic fibrosis. While this is technically feasible, meiotic recombination reduces the number of eggs that are useful for fertilization so that actually only about 1 egg in 4 will be appropriate to use. Because the polar body biopsy is only one third as efficient as direct embryo biopsy, this technique will probably have only very special applications, for example, in rare couples willing to undergo highly artificial technologies to avoid a conceptus with a genetic disease. Parenthetically, polar body biopsy is useless for determining the sex since all the polar bodies are female.

Comparison of Preimplantation Testing to CVS

Preimplantation testing associated with IVF is costly, approximately $10,000 with a 20% to 25% chance of pregnancy resulting in the young healthy couple. With CVS, there is 100% chance of pregnancy because pregnancy has already been achieved, and CVS, including the analytical methods, costs only 10% to 15% as much. Consequently, preimplantation testing is going to be limited to a few specialized centers and carefully selected patients.

Ethical Considerations

Preimplantation testing obviates abortion, which may be ethically more acceptable to some potential parents. It is possible that when couples are at high risk of having a child affected with disorders like cystic fibrosis, sickle cell anemia, and fragile X syndrome, which are not rapidly lethal, the family will prefer preimplantation testing to CVS.

Research for the Future

All of the preimplantation genetic procedures discussed here are currently research techniques. They are very new and will not be generally available for some time. The technique used to simply sex embryos by PCR is the only fairly reliable procedure at present.

PCR in single cells is technically demanding and of very limited success, especially with single copy genes. Use of a multicopy Y chromosome-specific probe is usually successful, since it is much easier to start out with 100 or so of the gene to conduct a successful analysis by PCR than to start with 1 or 2.

The development of enzyme assays to be utilized during later embryonic stages is receiving significant attention. Much more accurate data than that currently obtained are needed.

A technique that still has to be taken beyond the thinking stage is the recovery of fetal cells from maternal blood. The development of reliable methods for recovering fetal cells among large numbers of circulating maternal cells would open the way to noninvasive testing of the fetus.

Question-and-Answer Period

Q: Please discuss briefly ovum donation, fertilization, and implantation in patients with Turner syndrome.
A: That is now standard technology. It is very easy to obtain donor oocytes using nonsurgical techniques. Coordination is done of the cycles between the recipient and the donor either by exogenous hormonal control, natural synchronization, or freezing and thawing of the embryos. A number of pregnancies have been effected in this manner in patients with Turner syndrome.

Q: How long can one maintain an egg in the frozen state?
A: There is a big difference between freezing eggs and freezing embryos. Embryos can be frozen for many years, probably at least 5 or 10. Egg freezing is much less effective, much less reliable, and there have only been 3 or 4 pregnancies around the world from eggs that have been frozen. Our egg freezing attempts and those of most others have been disappointing, even though we have achieved many pregnancies with frozen embryos. There is some characteristic about the egg that makes it less suitable for freezing.

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In Future Issues

Rickets and Growth
by Dagfinn Aarskog, MD

Testicular Differentiating Factors: An Update
by Barbara MacGillivray, MD

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Growth Hormone Resistant Syndromes
by William Daughaday, MD

The Physiology and Pathophysiology of GH Secretion: An Update
by Alan Rogol, MD, PhD

Abstracts From the Literature

Human Growth Hormone Prevents the Protein Catabolic Side Effects of Prednisone in Humans

Four groups of normal adult males (n = 8, each group) were studied for the effects of glucocorticoid therapy on protein catabolism, using traditional N_2-balance and isotope dilution techniques. Administration of either prednisone (0.8 mg/kg/d), or recombinant human growth hormone (rhGH; 0.1 mg/kg/d), or both were studied for 7 days and compared with a control group.

Prednisone induced protein wasting, as determined by both methods, whereas rhGH alone resulted in positive protein balance, as compared with controls. Prednisone produced proteolysis, whereas rhGH increased whole body protein synthesis. rhGH plus prednisone at the doses used yielded results that were identical to the controls, implying that protein catabolic effects of glucocorticoids were prevented by the concomitant administration of rhGH.

Carbohydrate intolerance was observed only with the combined therapy, although hyperinsulinemia was noted in those receiving rhGH alone or prednisone alone.

The authors conclude that rhGH may have a distinct role in preventing the protein losses associated with administration of pharmacologic doses of glucocorticoids in humans. However, several potential drawbacks may exist to combined rhGH and prednisone therapy: (1) carbohydrate intolerance evolves within 8 days; (2) rhGH increases glomerular filtration rates, and recent attention has been drawn to the progression of renal failure associated with glomerular hyperfiltration; and (3) acromegaly may occur with long-term administration of rhGH. The authors caution that further studies using smaller pharmacologic doses are indicated before any attempts at therapy are made.

Horber FF, Marsh HM, Haymond MW. *J Clin Invest* 1990;86:265.

Editor's comment: *This study will be quoted and referred to as a classic study for many years. The authors identify the physiologic effects of prednisone and/or rhGH on protein, fat, and carbohydrate metabolism, and show how the concomitant administration of rhGH can counter the effect of prednisone on protein metabolism and multiply the effects of both on carbohydrate intolerance. Every reader who has any interest in endocrinology, metabolism, or nutrition should read this lengthy but superb article.*

Robert M. Blizzard, MD

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Differential Effects of Prednisone and Growth Hormone on Fuel Metabolism and Insulin Antagonism in Humans

Horber et al have previously reported *(J Clin Invest* 1990; 86:265) that recombinant human growth hormone (rhGH) therapy may have a role in preventing the protein losses associated with the administration of glucocorticoids in humans. This study is also reviewed in this issue of *GGH.* The present paper reports additional data regarding fat and carbohydrate metabolism obtained during the original study. Glucose and fat oxidation were determined utilizing isotopic dilution studies and indirect calorimetry. Four groups of normal adult males (N=8, each group) treated with (1) prednisone alone (0.8 mg/kg/d); (2) rhGH alone (0.1 mg/kg/d); (3) prednisone and rhGH; or (4) placebo were studied.

Fasting plasma glucose concentrations increased in the groups treated with prednisone and prednisone plus rhGH but fasting plasma insulin levels were higher only during combined treatment. Protein oxidation was decreased in the postabsorptive state in subjects receiving rhGH alone and increased in subjects receiving prednisone alone, but there was no difference in protein oxidation observed between the placebo-treated subjects and subjects treated with combined prednisone and rhGH. However, fasting fat oxidation was decreased in subjects treated with prednisone but not significantly increased in subjects treated with rhGH alone. The ratio of protein to fat oxidation was increased in subjects on prednisone alone, decreased in subjects treated with rhGH alone, and unchanged in subjects given the combined treatment as compared with controls. No differences in carbohydrate oxidation were observed among the different groups. The prednisone-treated subjects oxidized more protein but less fat than the controls, whereas the subjects treated with rhGH alone oxidized more fat but less protein than the controls. Subjects treated with both rhGH and prednisone oxidized more fat and less protein than controls.

The authors state that this study suggests that rhGH and prednisone induce insulin antagonism by independent mechanisms. Their conclusions are based on the observations that the increases in concentrations of glucose, insulin, and C peptide with combined rhGH and prednisone were synergistic. Secondly, prednisone alone decreased the plasma concentrations of free fatty acids and ketone bodies in the postabsorptive state but decreased fat oxidation and increased protein oxidation and plasma lactate and pyruvate in both the fed and fasted states. In contrast, therapy with rhGH alone increased fat oxidation, decreased protein oxidation, and had no effects on plasma concentrations of free fatty acids, ketone bodies, lactate, and pyruvate. Finally, although the combined treatment normalized protein and fat oxidation, the plasma concentrations of free fatty acids, lactate, and pyruvate remained elevated in the fed state. The authors further suggest that the mechanism for carbohydrate intolerance in subjects treated with prednisone alone appears to be a decrease in glucose clearance possibly related to a post-receptor defect. The mechanism for the inverse relationship between fatty acids and protein oxidation observed in this study remains unclear, but may be a result of reciprocal effects of the 2 drugs on enzymes that regulate the mobilization and oxidation of fatty acids and amino acids.

Horber F, Marsh H, Haymond M. *Diabetes* 1991;40:141-149.

Editor's comment: *This is an extremely detailed study that extends a previous report on the effects of rhGH and/or prednisone on protein homeostasis in normal adults. The authors have presented data suggesting that the insulin antagonism of rhGH and prednisone is probably caused by independent mechanisms, since it would appear that rhGH and prednisone reciprocally regulate the oxidation of protein and fat while decreasing the efficiency of glucose disposal. This paper along with the previously reported article should be read and studied together.*

William L. Clarke, MD

Growth Rate Reduction During Energy Restriction in Obese Adolescents

Amador et al studied the effects of energy restriction on growth and sexual development in a group of obese children who were participating in a multidisciplinary weight-loss program. Ninety-four children whose relative fat weight was determined to be above 25% but less than 40% in males and above 30% but less than 45% in females were studied. These children, aged 10.6 to 12.9 years, were all in Tanner stage II puberty and their body weight for stature was above the 97th percentile. The children were randomly classified into 2 groups: a control group in which energy intake was maintained (0.25 mJ/kg of expected body weight for height) and an experimental group in which energy intake was restricted to 30% of energy requirements (0.17 mJ/kg of expected body weight for height). All children participated in a program of physical activity, nutritional education, and behavioral modification. All subjects were measured and examined for height and stage of sexual development at the end of 6 months and again at 6 months following the intervention. Seventy-eight children completed the 1-year study.

No differences between the 2 groups were found with respect to Tanner stage, body weight, lean body weight, or fat body weight at the initiation of the study. However, after 6 months of therapy, puberty progressed at a significantly slower rate in the group with the lower energy intake. In addition, there was a significantly greater reduction in body weight in this group, with significantly greater loss of fat body weight than in the control group. The height velocity was also significantly slower in the energy-restricted group. During the subsequent 6 months, catch-up growth was evident in the energy-restricted group, but pubertal development continued to lag behind the group with less restriction of energy intake.

The authors suggest that the restriction of energy intake in early adolescence should be avoided in the dietary management of overweight early adolescent children. They suggest that a nonrestrictive diet with the addition of physical activities, nutritional education, and behavioral modification is a more appropriate method for achieving weight loss in this group.

Amador M, Ramos L, Morono M, et al. *Exp Clin Endocrinol* 1990;96:73-82.

Editor's comment: *This very interesting paper demonstrates once again the need for monitoring linear growth during weight-reduction therapy in children. Dietz et al (*AJCD *1985;139:75) demonstrated previously that diets with even a mild restriction of energy may be associated with a reduction in height velocity. The present study confirms this finding and, in addition, demonstrates a reduction in the tempo of pubertal development in children whose energy intake is restricted. They have demonstrated that a multidisciplinary program of exercise, education, and behavioral modification is exceedingly important to weight reduction programs in children.*

William L. Clarke, MD

Identification of the 64K Autoantigen in IDDM as Glutamic Acid Decarboxlyase (GAD)

An antigen previously found only in the beta cells of the islet cells here is reported also to be present in certain neurons that secrete gamma-aminobutyric acid (GABA) in the central nervous system (CNS). This antigen is identified as glutamic acid decarboxlyase (GAD), the biosynthesizing enzyme of the inhibitory neurotransmitter GABA.

Individuals with stiff-man syndrome (SMS) frequently have associated insulin-dependent diabetes mellitus (IDDM). Individuals with SMS have autoantibodies to 64K antigen at much greater titers than do most patients with IDDM. The authors found that the GAD antibodies in SMS were 10 to 200 times higher than those found usually in IDDM patients. A reference by Solimena et al in the *New England Journal of Medicine* in 1990 is quoted.

The authors used acceptable immunologic and microbiologic techniques to demonstrate that the 64K antigen in the beta cells and in the GABA-secreting neuron cells is the same. This finding is expected to motivate the creation of studies to elucidate the pathogenesis of IDDM and SMS, and to determine the mechanisms of generation of self-tolerance by the immune system and its failures.

The authors suggest there are components other than the GAD antibodies responsible for these diseases. For example, beta cells express major histocompatibility complex class I molecules whereas CNS neurons normally do not.

Baekkeskov S, Aanstoot HJ, Cristgau S, et al. *Nature* 1990;347:151-156.

Editor's comment: *The pancreatic 64K beta-cell autoantigen is a major target of autoantibodies associated with IDDM. The finding that this antigen is identical to that found in GABA-secreting neurons is a significant contribution. The details as presented in the abstract above are convincing. Those readers who are the least bit interested in the possible role of autoimmunity as a cause of IDDM can benefit by reading the entire article.*

Robert M. Blizzard, MD

Transplacental Passage of Insulin in Pregnant Women With Insulin-Dependent Diabetes Mellitus: Its Role in Fetal Macrosomia

Menon et al demonstrated that there is a cause other than the placental transfer of glucose from mother to fetus in the pregnant diabetic to account for the macrosomia that is frequently seen in the offspring. Specifically, placental transfer from the mother to fetus of animal insulin antibodies, which carry with them insulin, may account for some cases of macrosomia seen in the offspring of diabetic women.

The authors demonstrated a direct correlation between the insulin levels in umbilical cord blood and macrosomia. The 12 infants reported with macrosomia had significantly higher cord-serum concentrations of animal, human, and total insulin than did those infants who did not have macrosomia.

There were also significant correlations between birth weight and cord-serum concentrations of animal, human, and total insulin. There was no significant difference between the infants with macrosomia and those without in cord-serum insulin antibody activity, years of maternal diabetes before conception, maternal insulin dosage, maternal HbA_{1C}, incidence of respiratory distress syndrome, or low blood glucose concentration in the infant during the first 4 hours of life.

	Insulin in Cord-Serum*		
	Animal	Human	Total
Macrosomic infants	1,113 ± 32	2,726 ± 599	3,839 ± 840
Nonmacrosomic infants	402 ± 110	908 ± 163	1,309 ± 259
P	<0.05	<0.02	<0.02

*pmol/L

Menon RK, Cohen RM, Sperling MA, et al. *N Engl J Med* 1990;323:309.

Editor's comment: *Previously, the teaching has been that insulin does not pass from the mother to the fetus. The authors have shown the fallacy of that teaching. When antibodies to insulin are present, insulin crosses the placenta. The data implicate transplacental insulin as one of the accountable causes for macrosomia in infants of diabetic mothers. This is an important contribution to our understanding of fetal growth in the offspring of diabetic women. However, Dr. Robert Schwartz of Brown University commented in an editorial in the same issue of the* New England Journal of Medicine *that, usually, hyperinsulinemia is primarily fetal in origin in the fetus of the diabetic woman. Dr. Schwartz does not discount the role of insulin transferred via antibodies from mother to fetus in the production of macrosomia but does not readily accept it as the major mechanism in the production of macrosomia.*

Robert M. Blizzard, MD

Treatment of Short Stature in Renal Disease With Recombinant Human Growth Hormone

Rees et al report on their experience with the use of recombinant human growth hormone (rhGH) in 18 children with renal disease. These children were selected for study because they had the lowest height standard deviation scores (SDS) among children attending the chronic renal failure clinic and were failing to show catch-up growth despite theoretically optimum medical management. Three groups of 6 children each were studied: group 1 — prepubertal children with chronic renal failure (CRF); group 2 — prepubertal children with renal transplants; and group 3 — pubertal patients with renal transplants. All patients had attended the clinic for at least 18 months. Their height SDS and/or height velocities were >2 SD below the mean. All patients with transplants were receiving alternate-day prednisolone therapy. Height, weight, and pubertal status were assessed every 3 months. Bone age (BA) was assessed at the beginning of the study and after 12 months. Blood samples were evaluated every 3 months for electrolytes, BUN, creatinine, calcium, phosphate, bilirubin, albumin, hemoglobin, glucose, and glycosylated hemoglobin. Glomerular filtration rate was calculated at each visit. GH pulsatility was assessed by blood sampling (q15min) from 2000 to 0700 hours (8 PM to 7 AM) in all patients. Insulin-like growth factor I (IGF-I) levels, thyroid function, and parathyroid hormone concentrations were determined at the beginning and end of the study.

Each patient received rhGH at 30 U/M^2 per week (divided into daily doses) for up to 1 year (a median of 0.98 years [range, 0.25 to 0.99]). Two patients stopped GH therapy after 3 months (1 due to patient fear of the hypodermic needles and 1 due to noncompliance), and were not included in the analysis. As shown in Table 1 (page 14), mean height SDS increased significantly in group 1 and mean height velocity SDS increased significantly in all groups. BA, which was delayed in all subjects prior to treatment, remained delayed, and changes in BA and height SDS/BA were not significant. There were no correlations between the response to treatment and the severity of the CRF in group 1, or between the dosage of prednisolone and the growth responses in groups 2 and 3. In addition, there were no correlations between GH pulsatility and growth response in any groups since GH pulsatility was normal in all children prior to rhGH. Initial IGF-I levels were below the reference range for the prepubertal

Table 1
Treatment of Short Stature With Recombinant Human Growth Hormone

	Group 1 Prepubertal With Chronic Renal Failure (n=6)	Group 2 Prepubertal With Renal Transplants (n=6)	Group 3 Pubertal With Renal Transplants (n=6)
Prior to rhGH Rx			
Mean age (years)	7.7	12.1	15.6
Mean height SDS	-2.9	-3.3	-3.4
Mean height velocity SDS	-1.3	-2.0	-1.0
Mean BA delay (years)	-2.1	-2.5	-2.7
End of rhGH Rx			
Mean height SDS	-2.1*	-3.1	-3.2
Mean height velocity SDS	6.0*	0.6*	3.5*
Mean BA delay (years)	-1.6	-2.5	-2.9

* *P* at least < 0.05 compared with scores prior to rhGH Rx.
SDS, Standard deviation score; BA, Bone age.

children (group 1), but were within the reference range in groups 2 and 3. No significant changes in renal function were noted in any group. There was a significant increase in the mean glycosylated hemoglobin level during treatment; however, this increase did not result in values outside the normal range.

The authors point out that catch-up growth can occur in some children with CRF with correction of fluid, electrolytes, and acid-base balance, attention to energy and protein intake, and prevention or treatment of renal osteodystrophy. However, many children do not experience catch-up growth. With careful renal management, growth velocity may return to a normal rate, but catch-up growth is rare in the child over 2 years of age.

After the age of 2 years, normal growth rate is usual, but catch-up growth is rare. Catch-up growth can occur in prepubertal children after transplantation, although corticosteroids can interfere with the onset and progression of puberty and the pubertal growth spurt. Thus, the rates of growth observed by the authors far exceeded those previously achieved in their clinic by other means.

Rees L, Rigden S, Ward G, et al. *Arch Dis Child* 1990;65:856-860.

Editor's comment: *This paper presents data that can be added to the growing body of information concerning the effects of GH therapy in children with chronic renal disease (see also* GGH *Vol. 4, No. 3). As more data accumulates, it becomes clear that GH administration may be of clinical usefulness in some of these children. The failure of rhGH to increase height SDS in patients with renal transplants may be attributable to the effects of prednisolone or to the older ages (>12 years) of the children in these groups at the initiation of rhGH therapy. It is unfortunate that the authors were unable to match their patients to a control group. They have stated that their reason for not doing so was the number of variables involved (diagnosis, age of onset, severity of renal failure, etc). Thus, it would appear that a large-scale matched trial is needed before one can document the usefulness of GH therapy in CRF. In addition, such a trial may identify any adverse effects that might occur and the relative frequency of occurrence associated with rhGH therapy in chronic renal disease (ie, renal function deterioration, hypercalciuria, enhanced immune function, abnormal carbohydrate metabolism, mitogenic activity).*

William L. Clarke, MD

Pubertal Growth in Chronic Renal Failure

This paper analyzes the height growth of 15 boys and 14 girls with end-stage renal failure first studied before puberty and followed at 3- to 6-month intervals until growth ceased or nearly ceased. The height data were smoothed by the kernel estimation method, which is a form of moving average. The records were from Heidelberg, and the curves were compared with those from the Zurich Longitudinal Growth Study. This made possible a comparison with late normal maturers as well as with the average maturers in a normal growth study.

The start of the pubertal growth spurt was delayed by 2.5 years in both the girls and boys, and its duration and intensity were also very significantly reduced, with the mean height gain at around 50% of that observed in the late-maturing control group. However, mean height at the onset of the spurt was approximately the same as that in the late-maturing control group. The data indicate that most patients with end-stage renal failure occurring before or during puberty irreversibly lose growth potential. Renal transplantation did not consistently improve pubertal growth.

Schaefer F, Seidel C, Binding A, et al. *Pediatr Res* 1990;28:5.

Editor's comment: *This paper is particularly striking because of the use of the kernel estimation method, which, in my opinion, is currently the most advanced technique for analyzing growth curves. Since it is nonparametric, it is particularly applicable in cases of growth disorder, and this paper constitutes a real model for*

other research workers studying growth in chronic disease. It is interesting that in the patients with renal failure, puberty did not start until their height had reached virtually that of the controls when they started puberty; however, by this time height velocity was far below normal and the subsequent pubertal spurt was very much reduced. Such a fine analysis does require many measurements of height to be made during the growth period but results in a much better understanding of the dynamics associated with the disorder than has previously been possible.

James M. Tanner, MD

Hypersomatotropism in the Dysmature Infant at Term and at Preterm Birth

de Zegher et al report umbilical cord-serum growth hormone (GH) levels in a large group of small (<2.4 kg), appropriate (3.4 ± 0.1 kg), and large (>4.4 kg) infants born at term and in the cord-serum of prematurely born twins (28 to 36 weeks) in which both twins were appropriate for gestational age or 1 twin was appropriate and the other small for gestational age. The results demonstrate that appropriate and large infants have similar cord-serum GH concentrations (16.7 ± 1.0 ng/mL versus 16.5 ± 1.2 ng/mL respectively), but small infants have significantly elevated cord-serum GH levels (24.2 ± 1.8 ng/mL) when compared with either of the other 2 groups ($P < 0.001$). Serum GH concentrations in twins concordant for weight and appropriate for gestational age were similar, while GH levels were significantly higher ($P = 0.007$) in the smaller of twins discordant for weight.

The authors point out that the mechanisms underlying the elevations in cord-serum GH levels at birth are unclear, but that increased cord-serum levels of GH have been documented in both the human and ovine fetus when acidotic or hypoxic, in fetuses of undernourished ewes, in ovine fetuses undergoing surgery, and in ovine fetuses in conditions associated with growth retardation. Although GH is not known to influence fetal circulating insulin-like growth factor (IGF)-1 levels and is not essential for fetal growth, the authors state that the elevations in GH in the small-for-gestational age infant are likely to be related to insulin antagonizing actions or lipid metabolism. These data support the hypothesis that GH plays a homeostatic role in the late-gestational fetus in particular, and possibly in the metabolic adaptations to conditions associated with subnormal intrauterine growth.

de Zegher F, Kimpen J, Raus J, et al. *Biol Neonate* 1990;58:188-191.

Editor's comments: *This is a well-conducted study with appropriate controls that suggests that GH, although not necessarily involved in stimulating fetal growth, may play a very important role during the last trimester of pregnancy. Obviously, more research is needed to establish the significance of these findings.*

William L. Clarke, MD

30-Second Sampling of Plasma GH in Man: Correlation With Sleep Stages

The authors used a refined technique to draw 2 drops of blood every 30 seconds over an 8-hour period in 6 young male adults, following 24 hours of fasting. Growth hormone (GH) was measured on each sample. The accuracy was verified by comparing the GH concentrations in plasma and in whole blood. EEG recordings were used to correlate GH pulsatility with stages of sleep. GH pulses were analyzed by cluster analysis; GH secretion rates were determined by deconvolution analysis. Data analysis revealed the nocturnal pulse frequency to be 1.2 pulses per hour. If analysis had been done on blood samples drawn every 20 minutes, the number of identifiable peaks would have been 61% less, or 0.5 pulses per hour. Mean GH concentrations and secretory rates were significantly higher during stages 3 and 4 of sleep as compared with stages 1 and 2 and REM sleep. There was a close correlation of EEG-identifiable sleep and initiation of the GH secretory peaks (4.5 minute time delay). The authors suggest that normally there are major episodes of GH release (secretory episodes) that consist of multiple small pulses within each major episode.

Holl RW, Hartman ML, Veldhuis JD, et al. *J Clin Endocrinol Metab* 1991;72:854-861.

Editor's comment: *Working with these authors at the University of Virginia through the years has been both a pleasure and an enlightening experience. The method described for measuring GH in 2 drops of whole blood is remarkable — and it works! It is a research, not a diagnostic, tool. From the data, we can conclude that the number of GH pulses increases phenomenally based on the frequency with which the investigator analyzes GH. The reader needs to realize, however (as pointed out* by *Evans et al* Am J Physiol *1987;252:E459-E556), there are major secretory episodes, each comprised of multiple pulses. Sampling every 20 minutes permits identification of the majority of GH secretory episodes, but not pulses. For many physiologic and diagnostic studies, sampling blood and measuring GH in blood drawn every 20 minutes over 12 to 24 hours is adequate.*

Robert M. Blizzard, MD

August 25-29, 1991 30th Annual Meeting of the ESPE, Berlin, Federal Republic of Germany. Information: Dr. V. Hesse, ESPE Meeting 1991, Children's Hospital, Lindenhof, Goltlindenstrasse 2-20, 1130 Berlin, FRG.

September 15-19, 1991 6th International Congress of Auxology, Madrid, Spain. Scientific Information: Dr. M. Hernandez, Univ Autonoma de Madrid, Dept de Pediatria, Hospital del Nino Jesus, Avda Menendez Pelaya 65, 28009 Madrid, Spain. Fax: 34-1-574-4669. General Information: Compania Hispanoamerican de Turismo, Edificio Espana, Gran Via 88, 28013 Madrid, Spain. Tel: 34-1-247-5717. Fax: 341-541-2037.

September 23-27, 1991 Kabi Advanced Postgraduate Course on Growth and Growth Disorders, Stockholm, Sweden. Deadline for registration is July 1, 1991. Scientific information: Dr. P. Wilton. General information: Dr. S. Dahlskold/Dr. S. Renstad, Kabi Vitrum Peptide Hormones, S-11287 Stockholm, Sweden. Tel: 46-8-138-000. Tlx: 16338 Kupert S. Fax: 47-8-618-2019.

October 6-11, 1991 8th International Congress of Human Genetics, Washington, DC. Information: M. Ryan, ICHG, 9650 Rockville Pike, Bethesda, MD 20814 USA. Tel: 301-571-1825. Fax: 301-530-7079.

October 20-25, 1991 8th International Beilinson Symposium on Prediabetes, Jerusalem, Israel. Scientific Information: Prof Z. Laron, Institute of Pediatric and Adolescent Endocrinology, Beilinson Medical Center, Petah Tikva 49100, Israel. Tel: 972-3-9225108. Fax: 972-3-9229685. Information: Kenes, PO Box 50006, Tel Aviv 61500, Israel. Tel: 972-3-654571. Fax: 972-3-655674.

June 18-23, 1992 52nd Annual Meeting of the ADA, San Antonio, TX. Information: Meetings Department, ADA, 1660 Duke Street, Alexandria, VA 22314 USA. Tel: 703-549-1500, ext 330. Fax: 703-836-7439.

June 24-27, 1992 74th Annual Meeting of The Endocrine Society, San Antonio, TX. Information: Ann Singer, Meetings Manager, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 USA. Tel: 301-571-1802. Fax: 301-571-1869.

August 30 - September 5, 1992 9th International Congress of Endocrinology, Nice, France. Information: N.I.C.E. 92, c/o SOCF1, 14 rue Mandar, 75002 Paris, France.

September 7-10, 1992 31st Annual Meeting of the ESPE, Zaragoza, Spain. Information: Dr. A. Ferrandez-Longas, Endocrine Unit, Miguel Servet Children's Hospital, Paseo Isabel la Catolica 3, 50009 Zaragoza, Spain. Tel: 34-976-355-700.

June 3-7, 1993 4th Joint Meeting of the ESPE/LWPES, San Francisco, CA, USA. Information: Prof M. Grumbach, Dept of Pediatrics, Univ of California School of Medicine, San Francisco, CA 94143 USA. Tel: 415-476-2244. Fax: 415-476-4009.

June 9-12, 1993 75th Annual Meeting of The Endocrine Society, Las Vegas, NV, USA. Information: Scott Hunt, Director, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814 USA. Tel: 301-571-1802. Fax: 301-571-1869.

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AN UPDATE:

Growth Hormone Physiology and Pathophysiology

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Robert M. Blizzard, MD

This subject was presented in the premiere issue of *GROWTH, Genetics, & Hormones* (*GGH*) in 1985. Since then, much new information has become available concerning factors that stimulate the synthesis and release of growth hormone (GH), GH transport in serum by GH binding proteins (GHBPs), the structure of GH and related receptors, insulin-like growth factors (IGF-1 and IGF-2), IGF-binding proteins (IGFBPs), and the actions of GH. Placental GH (GH-V) has been identified in 2 distinct forms and preliminary information concerning its structure and action has been identified. The current presentation emphasizes information obtained regarding these phenomena since the previous review was written. Readers are encouraged to review the previous article (*GGH* 1985;1:1) to supplement the information presented here.

GH Synthesis and Release

As known for some time, GH synthesis by and its release from the pituitary somatotrope are under the regulation of GH-releasing hormone (GHRH) and somatostatin, or somatotropin release-inhibiting hormone (SRIH).[1,2] A pulse of GH is generated by the simultaneous rise of GHRH and decline in SRIH. The amount of GHRH released is believed to determine the amplitude of the GH peak, and the frequency and duration of the GH secretory event is primarily under SRIH control.[1,2] These rhythmic patterns may be intrinsic to the hypothalamus or may be under control of higher neural oscillatory mechanisms.

The hypothalamic hormones, GHRH and SRIH, are regulated by neuromodulatory biogenic monoamines and other neuropeptides (eg, galanin), although some of these factors such as dopamine, norepinephrine, and epinephrine may also act directly on the somatotrope. Cholinergic agonists and cholinesterase inhibitors generally enhance GH secretion, particularly in response to GHRH and other stimuli, while cholinergic antagonists inhibit GH secretion by GHRH and other stimuli. These data suggest that somatostatin secretion is primarily regulated by cholinergic mechanisms: cholinergic antagonists stimulate SRIH production or action and agonists inhibit SRIH production or action.

Alpha-adrenergic agonists stimulate GH release via GHRH and beta-adrenergic agonists inhibit GH secretion in vivo, presumably through increased release of SRIH. In contrast, atenolol, a beta-adrenergic blocking agent, enhances GH release when pharmacologic stimuli for GH release are given. Dopamine agonists both stimulate and inhibit GH release depending upon specific conditions. For example, L-dopa (which crosses the blood-brain barrier) will cause the release of GH in normal individuals, but may inhibit GH release in acromegalic patients.

These in vivo effects may relate to conversion of dopamine to norepinephrine, since dopamine inhibits GH release following arginine administration or the induction of hypoglycemia in some acromegalic patients. This is a logical assumption since norepinephrine blocks the release of GH to arginine or insulin-induced hypoglycemia.

In This Issue

Serotonin and its precursors, tryptophan and 5-hydroxy-tryptophan, also induce the release of GH in vivo, but the mechanism is uncertain.

Since 1985, 2 other stimuli have been described. A GH-releasing peptide (GHRP), a hexapeptide (his-D-trp-ala-trp-D-phe-lys-NH_2),[3,4] has been developed synthetically but is not homologous to the GHRH-related peptides of 40 or 44 amino acids. Its activity in humans is not diminished by SRIH and its ability to cause the release of GH when submaximal amounts of GHRH are given is at least additive and possibly synergistic. The role of this peptide or its native homologue, if such exists, has not yet been defined.

Galanin[5,6] is a naturally occurring neuropeptide present in considerable amounts in the median eminence of the hypothalamus. It causes GH release under appropriate conditions; however, its physiologic role is poorly understood. In humans, the administration of porcine galanin is followed by GH release, and it enhances by 3-fold the GH release in response to GHRH.[5] Its effect is thought to be indirectly mediated by epinephrine acting on GHRH neurons. Galanin is considered to control pulsatile GH secretion by decreasing somatostatin inhibitory tone.[6,7] Neither GHRP nor galanin appears at this time to be as important as GHRH in producing GH release, although the presence of galanin in the median eminence suggests an innate biologic function.

Other aspects of GH secretion that have been clarified in the past 6 years include the effects of estrogen and testosterone. At even very low doses of gonadal steroid hormones, the quantity of GH secreted over a 24-hour period is increased by 2 to 3 times the amount secreted in their absence. These increases occur as a result of an increase in the pulse amplitude of the GH secretory episodes and not by increasing the frequency of peaks (approximately 7 or 8 per day) in both the prepubertal and pubertal periods.[8] Also included in new information is the observation reported recently by Martha and coworkers[9] and Veldhuis and colleagues[10] that GH secretion is inversely related to body mass index (wt/ht^2). This observation may provide one explanation for extremes of GH secretion among normal individuals who, except for differences in body mass index, are indistinguishable.

GH Transport in Serum

GH is transported in serum attached to binding proteins, the major one of which is closely related to the GH receptor.[11] This binding protein is identical to the extracellular domain of the receptor, and appears to be generated either by alternative splicing of the receptor mRNA with direct extrusion of the protein into serum or by a trypsin-like cleavage of the extracellular component of the receptor in vivo. Approximately 45% of circulating GH is bound to this high affinity protein, and 5% is bound to a low affinity GHBP of 100 kd molecular weight. Approximately 50% of the GH remains unbound, or "free." Circulating levels of GHBP are low in the fetus and newborn, but increase over time, especially during the pubertal growth spurt. GHBPs[11] and the GH receptors[12] are absent as a result of partial gene deletion[13] in children with GH insensitivity (Laron type dwarfism) who have a GHD-like phenotype and fail to respond to GH administration. The exact roles of these binding proteins are unknown, but they may decrease the rate of degradation of GH or modify the availability of GH in some other way.

GH Receptor and Related Receptors

The GH receptor is a member of a family of 3 related straight-chain polypeptides with specificity toward GH (somatogenic), prolactin (lactogenic), and chorionic somatomammotropin or placental lactogen. The clinical significance of the ability of GH to affect the prolactin receptor and prolactin to act at the somatogenic receptor is not known. Some patients with acromegaly (approximately 25% to 50%) will have mild hyperprolactinemia, but only a small percentage will have galactorrhea. Whether this biologic effect is due to GH acting at the lactogenic receptor, due to prolactin itself, or due to some other mechanism has not yet been determined. Other members of the superfamily include receptors for some of the interleukins, granulocyte-macrophage colony—stimulating factor, and erythropoietin. All are single-chain glycoproteins with homology in a 210 amino-acid sequence with the extracellular, ligand-binding region of the amino terminus. This segment is attached to a variable length intracellular component of the receptor by a highly homologous transmembrane segment of 22 amino acids.

IGF and IGFBPs

IGF and IGFBPs are closely associated. Both IGF-1 (somatomedin C) and IGFBP-3, the major BP for IGF-1, increase with increasing GH production and fall with decreasing GH production. Recent data[14] indicate that IGFBP-3 controls the bioavailability of IGF-1 and IGF-2. Therefore, IGFBP-3 appears to control the action of IGF-1 as well as GH secretion.

These BPs, which now number at least 6, may be classified on the basis of (1) the amino-acid sequences, (2) molecular weight, or (3) immunoreactivity. Three of these are shown in Table 1. IGFBP-3, the acid-stable component of the 150 kd complex, probably is a major factor in growth regulation and GH secretion. It is glycosylated and consists of a 53 kd and a 47 kd component. Serum levels are high in the fetus, but drop shortly

after birth and then increase slowly until late prepuberty or very early puberty, at which time the values subsequently increase approximately 3-fold. IGF-1 levels increase concomitantly, as does the quantity of GH secreted with each secretory episode in adolescent boys at Tanner stage III or IV of pubertal development.[8] Peak values occur earlier in pubertal females. The levels of IGF-1 and IGFBP-3 are high in GH hypersecretory states, eg, acromegaly. The role of IGFBP-3 in respect to IGF-1 remains to be elucidated. From the clinical aspect, however, it is now abundantly clear that acid extraction of IGF-1 from its binding protein and measurement of the resultant IGF-1 more clearly reflects the growth-promoting effect than measurement of the IGF-1 concentrations using the nonextracted procedure.

IGFBP-1 is generally independent of GH, is found normally in amniotic fluid (placental source), is high in fetal blood, and declines immediately after birth and falls further throughout infancy, childhood, and adolescence. Since IGFBP-1 and IGFBP-2 are GH-independent, and because their function may be minimal in respect to growth, they are not considered further.

GH and IGF-1 Actions

GH, IGF-1, and other growth factors work together to promote cartilage and bone growth, as described separately in *GGH* 1990;6:2 by Horton and by Mohan. Many factors have been shown to influence 1 or more aspects of this scheme through endocrine, paracrine, and possibly autocrine mechanisms. GH has a dual effect of epiphyseal cartilage growth and differentiation of cartilage cells as well as generation of IGF-1.[15,16] The proximal zone, close to the bony epiphysis, consists of a narrow band of germinal or stem cell chondrocytes. GH preferentially stimulates differentiation of these prechondrocytes while IGF-1 stimulates the clonal expansion of the more differentiated cells in the distal proliferative zones.

The demonstration by Walker et al[17] that IGF-1 produces both positive nitrogen balance and hypercalciuria in patients with absent GH receptors indicates that GH itself is not necessary for the metabolic actions of GH except to generate IGF-1, but does not exclude the need for GH to promote chondrogenesis.

New data also have accumulated concerning the complex pathway of direct GH action to generate protein synthesis and growth. Three messengers or messenger systems appear to be involved in at least certain cell systems. GH is the first messenger, and by binding to its receptor, activates the second messenger system, diacylglycerol and protein kinase C. The oncogene, C-*fos*,[18] may play a role as a nuclear switch or as a third messenger in some signal transducing systems to activate transcription of appropriate genes to influence the biologic pathway of GH action.

Placental GH (GH-V)

Since 1985, several investigators have determined the structure and role of "placental" GH, the natural product of the GH-V (GH variant) gene. This protein is expressed primarily by the placenta.[19] Daughaday and colleagues[20] have demonstrated large quantities of this GH variant (20 to 30 times the mean GH level of nonpregnant women) late in pregnancy at a time when essentially no pituitary GH was demonstrable. IGF-1 levels do not increase into the acromegalic level during pregnancy, reflecting either inhibitors of IGF-1 generation by the large amounts of estrogen or failure of the GH-V to activate the second messenger system(s), despite the known effect of GH-V binding to the somatogenic receptor.

Two distinct species (hGH-V and hGH-V2) are synthesized by the placenta.[21] The first is a 22 kd and the second a 26 kd protein. The hGH-V2 protein differs from the hGH-V protein in the location of its intramolecular disulfide bonds. The hGH-V2 protein is secreted by the syncytiotrophoblast. The configuration of residues suggests that this hGH variant may be an integral membrane protein. hGH-V2 constitutes one third or more of total hGH-V mRNA. Interestingly, the predominant hGH-V or 22 kd placental protein differs by only 13 amino acids from the 22 kd pituitary hGH-N gene product.

MacLeod et al[22] demonstrated that the hGH-V variant is a biologically active somatogen and lactogen. hGH-V binds efficiently to both somatogenic and lactogenic receptors, but with a 7.4-fold greater specificity for the somatogenic receptor than does hGH-N. Both hGH-V and hGH-N produced similar weight gain at similar doses in hypophysectomized rats, but the mitotic response of the lactogen-inducible *N*b2 cells was significantly less for hGH-V. The comparable somatogenic, but lower lactogenic, bioactivity of hGH-V relative to hGH-N parallels the receptor binding profiles of the 2 hormones and suggests hGH-V has the potential to perform a unique role during human gestation.[23]

Summary

GH appears to be even more important and more complex in its physiologic actions than was imagined in 1984 when the first review of this topic was written for the premiere issue of *GGH*. GHRH as a potential therapeutic agent has been temporarily shelved awaiting a depot formulation. GHRP and galanin have been recognized and continue to be studied although their direct therapeutic role can only be speculated. The binding proteins for GH and

the IGFs are now recognized as potent players in the physiologic arena of GH and IGF action. IGF-1 is an effective therapeutic agent in patients who have absent receptors for GH. Possibly of equal importance, a new GH regulatory system of the placental-fetal unit has been recognized and is being studied.

We never anticipated in 1984 when the previous article was written that so much would be learned in the short period of 7 years. We anxiously await the passage of another 7 years when, hopefully, we will have the opportunity to update you regarding GH physiology and pathophysiology in volume 14 of *GROWTH, Genetics, & Hormones.*

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Limb Lengthening: Past, Present, and Future

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Abstracted by
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Introduction

In November 1990 at the National Cooperative Investigators Meeting of Genentech, Inc, Dr. David Rimoin gave an exciting presentation regarding the current and prospective status of leg lengthening to increase the height of individuals with short stature of skeletal etiology. This manuscript is an abstract of his presentation.

Limb lengthening has been a topic of increasingly developing interest over the past 2 years when reports first came out of Europe that it is possible to lengthen the limbs of chondroplastic children. Previously, the technique was used to correct limb asymmetry due to polio, neurologic disease, and some congenital anomalies.

Wagner in Germany established one of the early techniques, which entailed breaking the bone surgically, performing an open osteotomy, cutting the periosteum, and using an external fixator and telescoping rod to stretch the tissues, which pulled the fracture site apart. Subsequently, he put in a metal plate, filled the area with chips, and it would heal. He operated upon achondroplastic individuals. He claimed that some needed up to 50 operations and that the complications were numerous. This technique has been abandoned for dwarfed individuals.

Ilizarov, an orthopedic surgeon working in a small institute in Siberia, was also one of the first to apply limb-lengthening techniques to dwarfs. In contrast to Wagner, Ilizarov utilized "bloodless surgery," as all the incisions were small percutaneous cuts. Utilizing little scalpels and chisels, he broke the bone percutaneously, put on circular fixators, and then gradually stretched the extremities. This limb-lengthening procedure involved slow, controlled distraction of the callus during its formation. Interestingly, histologic examination revealed that the new bone was very organized, longitudinally oriented, and that the organic matrix was capable of mineralization, which began to occur in the first few days. The new bone that started in the medullary canal ossified rapidly and underwent corticalization after stretching was stopped. Perfectly normal appearing bone was present once the procedure was completed. Complications of extended limb lengthening were significant, however, and only a

rare patient did not have at least 1 complication; and most had numerous complications.The real question was pointed out by Dr. Rimoin, who asked, "Are these complications worth what one gains?" Muscle contractures, neurologic compromise because of the stretch of the nerves, vascular complications, joint stiffness, problems at the pin site, and a variety of psychologic problems may occur.

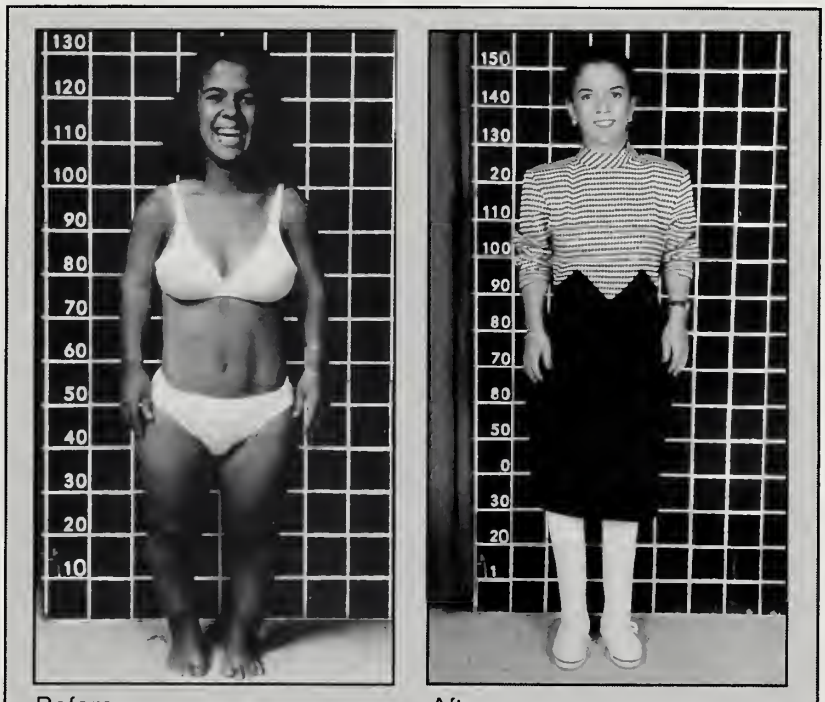
A variety of techniques have been developed subsequently by Villarubias in Barcelona and a number of Italian investigators who use external distraction with only 1 bar rather than the circular fixator developed by Ilizarov. Dr. Rimoin stated that he now is convinced that these techniques are worth trying–particularly the Spanish technique. Villarubias lengthens both tibias initially, and both femurs are subsequently lengthened. This is in contrast to the techniques used by the Soviets and Italians, in which a femur and a tibia may be lengthened on the same or opposite sides. Villarubias's technique also differs in that distraction is started within a few days after the initial surgery rather than waiting a longer period. His technique also differs in that he does not permit weight bearing during stretching, as opposed to the weight bearing required by the Soviet and Italian procedures. Rimoin's observation is that there is much less discomfort and little pain in patients who utilize the Spanish technique. Villarubias also does prophylactic tenotomies while he is doing the initial surgery. This consists of splitting the edges of the tendons, thus preventing the contractures that have occurred with the other techniques. By his technique, the limbs are stretched approximately 1.2 mm per day. Patients are kept in the hospital for only a brief period (3 to 5 days) and are then allowed to go to school in a wheelchair.

It is of great importance not to disturb the blood supply, which maintains the callus and allows it to heal rapidly. A few patients will have as much as a 30% reduction of ankle mobility and/or premature consolidation of the fibula, which may give obvious deviations of the extremity; about 2% have had to have repeat tenotomies. These complications are mild compared with those incurred with the other techniques.

Once the tibias have been lengthened, attention is focused on the femurs. Percutaneous tenotomy of the adductors is done early, and screws are put in the bones asymmetrically so that a rotational osteotomy is accomplished. A percutaneous tenotomy of the internal rectorus also is done, which relieves the tight muscles and tendons. No flexion of the knees is allowed during this time because the knee may be dislocated during femur stretching if it is placed in a fixed position. Patients are confined to wheelchairs.

Before initiating studies in Los Angeles, Dr. Rimoin visited Barcelona to observe the technique of Villarubias. Subsequently, Rimoin sent 2 orthopedic surgeons, Drs. J. Isoaeson and W. Oppenheim, who are his collaborators, to learn the technique in Barcelona. As of the time of Rimoin's report in November 1991, 5 patients had been treated in Los Angeles. Two essentially had completed their leg lengthening. In Figure 1, the end result of a patient with achondroplasia who had such leg lengthening of both the tibias and femurs is demonstrated. This patient had a final height of 61.5 in. Utilizing this procedure, up to 12 in of increased growth can be anticipated. Dwarfs can be made into normal-sized individuals. Amazing to all observers is that the achondroplastic patients who have severe lordosis end up with essentially no lordosis when the Villarubias technique is applied. The coccyx is pulled down vertically, which reduces the lordosis markedly (Figure 2, page 6).

Dr. Rimoin states that the technique is still difficult to perform but it can achieve results. It must



Before After

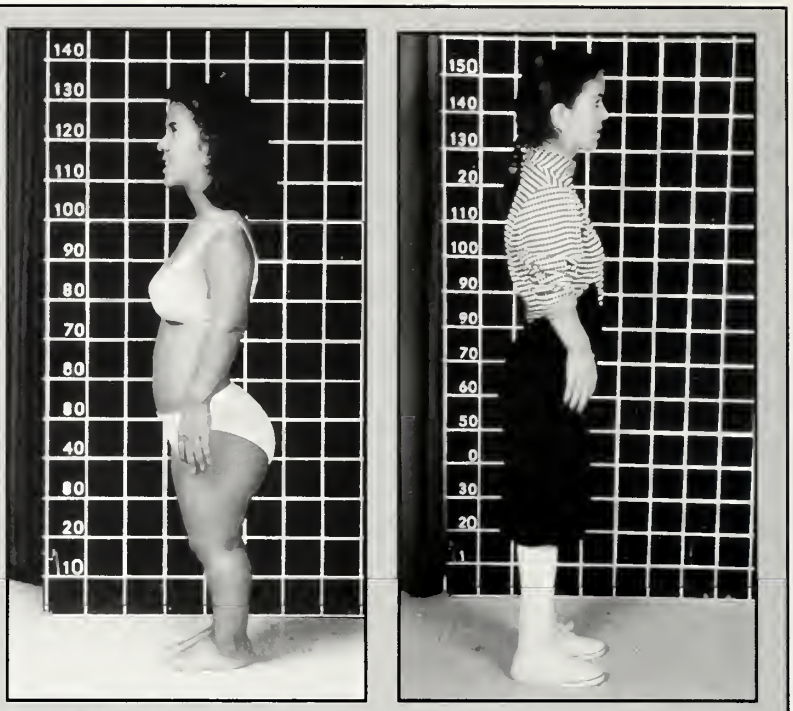
Figure 1: An achondroplastic girl pre- and post-leg lengthening. The final height was 61.5 in (156.2 cm.)

be done with a team whose participants are comfortable dealing with little people, that has broad surgical expertise, and comprises neurologists, geneticists, physical therapists, and psychiatrists or psychologists, all of whom are willing to be involved extensively.

Rimoin emphasized that one of the reasons that achondroplastic patients are such ideal candidates is that they have excess soft tissue in association with their short limbs. Because the soft tissues are in excess, and the blood vessels are long and tortuous, the soft tissues stretch readily and lengthening is primarily of the bone and not of the soft tissues.

What is the best time to perform the procedure? Ilizarov does it any time after 6 years of age. Villarubias does it any time after 10 years of age. Rimoin and his collaborators have decided upon 14 to 20 years. The emotional maturity that develops during the teenage period is important in helping the patients cope.

Rimoin thinks that in most instances it may be unwise to do the procedure before age 14 or 15. He emphasizes that it should be the child who makes the decision and not the parents, and he emphasizes very strongly that the technique should be done in centers with extensive capabilities and experience by the team members. Dr. Rimoin pointed out that leg lengthening was less expensive in treating achondroplasia on an inch-for-inch basis than growth hormone treatment in growth hormone-deficient patients. He emphasized that we all should be looking with great interest upon this technique, but that only a few should be trying it at this time.



Before After

Figure 2: The lordosis in this patient was remarkably decreased by surgery.

Support Groups for Individuals With Growth Problems and Their Families

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Judith G. Hall, MD

Genetic support organizations for families of children with growth disorders have increased steadily since the 1960s. Support groups help individuals with genetic disorders and their families discover that they are not alone and can be helped by others affected. Ideally, a strong partnership between the support group members and interested health professionals enables the organization to meet its goals and sensitizes health care providers to the needs of those they are serving. Genetic support groups are gaining recognition as an important component of ongoing health care for individuals and families with genetic disorders. To enhance the effectiveness of these efforts, it is essential that physicians providing care to children with growth problems be familiar with these voluntary organizations.

Group members share information on effective ways to cope, often working with health professionals to stimulate and fund research and to educate themselves and the public on a specific genetic disorder. Members also cooperate to effect changes in discriminatory laws and to obtain federal/state

funding for people with similar genetic disorders.

Below you will find several of these support groups listed for your reference.

Two national "umbrella" organizations have been formed to encourage greater public and professional awareness of these resource groups and to help extend group services when appropriate. The NATIONAL ORGANIZATION FOR RARE DISORDERS (NORD) was created in response to the unavailability of orphan drugs from the pharmaceutical industry for the treatment of rare disorders due to the economic infeasibility of producing these agents. NORD fosters communication among agencies and governmental and scientific communities, promotes scientific research on rare disorders, and represents people with rare disorders (when an appropriate organization does not exist), by putting them in touch with others through NORD's computer data base. The mailing address for NORD is: PO Box 8923, New Fairfield, CT 06812; (Telephone 1(800)999-6673).

Another national network of voluntary organizations is the ALLIANCE OF GENETIC SUPPORT GROUPS, formed to unify efforts of genetic support groups in educating the public about genetic disorders and to strengthen relationships between consumers and professionals. A recent survey of genetic support groups confirmed the value of professional services, such as genetic counseling, and recognized support groups as an important extension of health-care provider services. The ALLIANCE is trying to improve the availability and appropriateness of genetic services by developing model programs to meet needs identified by the membership. The mailing address is: ALLIANCE OF GENETIC SUPPORT GROUPS; 1001 22nd St, Suite 800; Washington, DC 20037; (Telephone 1(800)336-GENE).

LITTLE PEOPLE OF AMERICA (LPA) was founded in 1957 by Billy Barty, a little person himself and a Hollywood actor. LPA was established to assist members in acquiring the skills needed to become participating members of society, with an emphasis on education and employment. It is a unique organization administrated by affected individuals, rather than their parents. While one important aspect of LPA is to facilitate social interaction, recently the support group has incorporated educational/therapeutic workshops and panels into its annual convention format.

LPA has 12 regional, districts, with local, regional, and national meetings occuring regularly. Today LPA has more than 4,000 members, each meeting the membership criterion of being 4 ft 10 in or less. Active subgroups have been formed for parents, teens, and young adults, with committees and workshops on subjects including careers, nutrition, adoption, social attitudes, and exercise and fitness. (The DWARFS' ATHLETIC ASSOCIATION has participated in the Special Olympics.) The LPA Foundation obtains and distributes funds for vocational training, scholarships, and medical/scientific research. The Medical Advisory Board serves as a resource for medical care and advice, and the review of research projects for ethical and scientific merit. LPA publishes some excellent reading material (eg, *The Idea Machine* providing tips for easing daily living and *My Child Is a Dwarf*, a booklet for parents) and a newsletter which is distributed nationally.

Physicians, allied health professionals, and families are encouraged to write directly to LPA National Headquarters; PO Box 9897; Washington, DC 20016 or to its Canadian counterpart, Little People of Canada; PO Box 453; Abbotsford, British Columbia V2X 275, Canada for additional information.

THE HUMAN GROWTH FOUNDATION (HGF) was organized in 1965 by parents of children with severe growth problems. Largely through the efforts of the HGF, growth hormone therapy became available. From its inception, the HGF has worked to support basic clinical research pertaining to growth disorders. The 3 main goals of HGF are to disseminate information about growth disorders, to encourage the development of parent support groups at the local level, and to oversee a grants program to support growth disorders reseach. HGF publishes a monthly bulletin, *Fourth Friday*, in addition to a periodic newsletter.

HGF has also produced excellent informational booklets on growth problems including achondroplasia, Turner syndrome, intrauterine growth retardation, short stature, and dwarfism. These booklets are an important resource to parents and affected children. Inquiries may be addressed to Deborah Swansburg, Esq; Executive Director; HUMAN GROWTH FOUNDATION; 7777 Leesburg Pike, Suite 202 S; Falls Church, VA 22043; (Telephone 1(800)451-6434).

Societies for Specific Disorders

Several voluntary organizations have been established for families and individuals with short stature due to a specific disorder. Among these are the TURNER'S SYNDROME SOCIETY OF THE UNITED STATES, the OSTEOGENESIS IMPERFECTA FOUNDATION, the MUCOPOLYSACCHARIDOSES SOCIETY (MPS, Inc), the ASSOCIATION OF CHILDREN WITH RUSSELL-SILVER SYNDROME (ACRSS, Inc), and the PRADER-WILLI SYNDROME ASSOCIATION (PWSA).

Individuals with Turner syndrome have a special set of concerns, in addition to those associated with short stature. The TURNER'S SYNDROME SOCIETY OF THE UNITED STATES addresses these

concerns. The office address is: 768-214 Twelve Oaks; 15500 Wayzata Boulevard; Minnetonka, MN 55391; (Telephone 1(612)475-9944). The US group has educational booklets for patients/families and physicians available.

1. *Turner Syndrome: A Guide for Families* (P. Reisner, RN, and L. Underwood, MD).
2. *Turner Syndrome: A Guide for Physicians* (R. Rosenfeld, MD).

The CANADIAN TURNER'S SYNDROME SOCIETY has produced an excellent videotape and publishes an informative newsletter every few months. A booklet prepared by the Society, *The X's and O's of Turner's Syndrome*, can be obtained by writing to: TURNER'S SYNDROME SOCIETY; York University; Administrative Studies Building, No.006; 4600 Keele St; Downsview, Ontario M3J 1P3, Canada.

The OSTEOGENESIS IMPERFECTA FOUNDATION distributes information about osteogenesis imperfecta, provides moral support, and funds research. The Foundation also publishes a quarterly newsletter, *Breakthrough*. Although not all individuals with osteogenesis imperfecta are short statured, many do have medical and social problems. All types of osteogenesis imperfecta appear to be linked to genetic collagen abnormalities. Complications include frequent bone fractures, dental anomalies, and deafness. For further information about this disorder or the support group, contact: OSTEOGENESIS IMPERFECTA FOUNDATION; 12807 W. Hillsborough Avenue, Suite G-10, Tampa, FL 33635; (Telephone 1(813)855-7077).

The mucopolysaccharidoses and mucolipidoses are rare hereditary disorders with enzyme deficiencies in which abnormal compounds collect in the cells of various body tissues. Most of these disorders result in short stature and are associated with a variety of other problems. The NATIONAL MUCOPOLYSACCHARIDOSES (MPS) SOCIETY, INC is dedicated to serving parents through support, networking, physician referrals, professional and public education, and fund raising to support MPS research. The MPS SOCIETY is located at 17 Kraemer St; Hicksville, NY 11801; (Telephone 1(516)931-6338) and the CANADIAN SOCIETY for MPS is located at 382 Parkway Blvd; Flen Flon, Manitoba R8A 0K4, Canada.

THE ASSOCIATION OF CHILDREN WITH RUSSELL-SILVER SYNDROME (ACRSS) has recently been formed for families of children with Russell-Silver intrauterine growth retardation. Contact can be made through ACRSS, Inc, c/o Jodie Swain; 22 Hoyt St; Madison, NJ 07940; (Telephone 1(201)377-4531).

The PRADER-WILLI SYNDROME ASSOCIATION (PWSA), established in 1975, provides educational materials and supportive services to parents and professionals. It publishes a bimonthly newsletter, with a catalogue and audiovisuals available upon request. For additional information contact: Marge A. Wett; Executive Director; PRADER-WILLI SYNDROME ASSOCIATION; 6490 Excelsior Blvd, E-102; St Louis Park, MN 55426; (Telephone 1(612)926-1947).

International Support Groups

Recently, support groups for short statured persons and their families have developed in several countries. One of the early groups, ASSOCIATION FOR RESEARCH INTO RESTRICTED GROWTH (ARRG), founded in 1970 in Great Britain, is now called the RESTRICTED GROWTH ASSOCIATION. (For information contact: Miss Pam Rutt; 61 Lady Walk; Maple Cross, Rickmansworth; Herts, WD3 2YZ England). Other active support groups exist throughout Europe. Recently, the INTERNATIONAL GROWTH FEDERATION emerged to represent individuals from a variety of countries interested in growth disorders. For additional information about the INTERNATIONAL GROWTH FEDERATION, contact the HUMAN GROWTH FOUNDATION office in Virginia. (See above.)

This list of short stature support organizations is not all inclusive, but is intended to alert physicians to the availability of these resources for patients and families. It is important for patients and their families to be aware of the availability of reliable educational information and support groups, and for physicians to participate in this educational process. Lay groups also need the support of the medical profession to work effectively in dealing with the problems associated with short stature.

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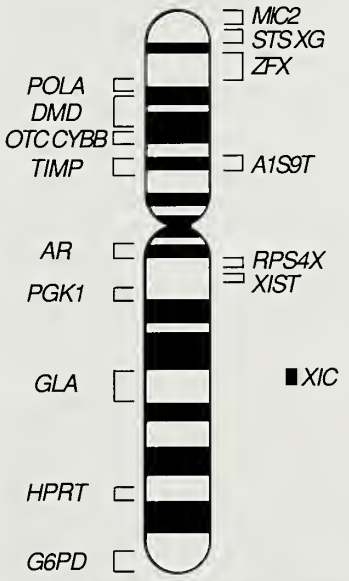
Review and Editor's Comment: X Inactivation of the X Chromosome Is Not Complete

A series of papers recently published present concurring data indicating that the inactivated X chromosome is not completely inactivated. There are now known at least 4 areas of Xp and 2 areas of Xq of the inactivated X chromosome that are active. Those genes known to be subject to X inactivation, and those known to escape X inactivation on the X chromosome, are depicted in Figure 1. One of the 2 areas on Xq encompasses the inactivation center, which is believed to be responsible for inactivation of 50% of the X chromosomes. Intriguingly, current thinking regarding this center is that this area is active on the inactive X chromosome but inactive on the active X chromosome. This area on the inactive X chromosome, known as the X inactive specific transcript (XIST), produces a transcript, whereas the same area on the active X does not. This area is an area for a candidate gene that could be involved in influencing the process of X inactivation.

The importance of this phenomena is considered further in the references listed below. The readers are encouraged to review these articles in detail, as Ohno's law of the constancy of the genetic material of the mammalian X chromosome is now being challenged by Watson et al after having been accepted for almost 25 years. The field is also intriguing as rare families manifesting female-to-female transmission of X-linked traits such as hemophilia B may have a co-inherited defect in the X inactivation center (XIC) resulting in the exclusive inactivation of the normal chromosome. XIST also may be involved in the phenotype of X-chromosome disorders such as Klinefelter and Turner syndromes. Deletion mapping in 46,XY Turner females has refined 1 probable Y chromosome localization to a 90 kb stretch between the possible sex determining gene, SRY, and the more proximal ZFY gene.

Judith G. Hall, MD

Figure 1



Map of the human X chromosome depicting genes and their inactivation state.

Davies K. *Nature* 1991;349:15-16.

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Review: Magnetic Resonance Imaging in Patients With Hypothalamic and/or Pituitary Disorders

In recent years, magnetic resonance imaging (MRI) has developed as an extremely useful tool to evaluate hypopituitarism, sexual precocity, diabetes insipidus, and other endocrine entities occurring in children. Pellini et al report the results of MRI in 30 growth hormone deficient (GHD) children, aged 10.1 ± 3.5 years, who were considered after routine investigation to have idiopathic GHD, and compared these with data from 15 healthy age-matched controls. Eighteen patients had isolated GHD and 12 had multiple deficiencies. The sellar and/or the hypophyseal volumes were significantly reduced in the GHD patients, without correlation with auxologic or endocrinologic findings. Twenty patients (all 12 with multiple deficiencies and 8 of the 18 with isolated GHD) had an abnormality of the pituitary stalk. In 18 cases, the bright spot indicating the neurohypophysis was dislocated to the distal part

of the stalk, but there was no change of water balance.

Cacciari et al report a study done in 70 patients with GHD and short stature, and in 6 with hypogonadotropic hypogonadism, 4 with isolated diabetes insipidus, and 21 with true central precocious puberty. Of the 70 patients, 23 had multiple anterior pituitary deficiencies and 42 had isolated GHD. The remaining 5 had anterior and posterior pituitary hormone deficiencies. The patients with multiple pituitary deficiencies had morphologic findings consistently involving the stalk and posterior lobe. Only 5 of 42 (12%) patients with isolated GHD had abnormalities of the sella.

In contrast, Pellini et al report abnormalities in 8 of 20 patients with isolated GHD. This group suggests that if a defect is found by MRI in a patient with isolated GHD, the patient very probably is at increased risk to develop multiple hormone deficiencies of the anterior pituitary. Stanhope et al previously reported in *Acta Paediatr Scand* 1986;75:799 that 64 of 77 patients affected by either isolated GHD or GHD with other tropic hormone deficiencies had pituitary hypoplasia.

Of significance also was the finding by Pellini et al that substitution therapy with GH does not induce shrinkage of the pituitary as determined by MRI, thus suggesting that a hypoplastic pituitary in a patient treated with GH probably can be interpreted as having been present before GH treatment was given.

Cacciari et al reported that patients with diabetes insipidus frequently had an absent or ectopic posterior gland and the bright spot was absent. They also reported that among 21 patients with precocious puberty, the MRI studies in 17 were normal, 1 was questionably abnormal, and hamartomas were observed in 3. All the latter were in children less than 2 years of age. No lesions were found in the 6 patients with hypogonadotropic hypogonadism.

Pellini C, et al. *Eur J Pediatr* 1990;149:536-541.

Cacciari E, et al. *Arch Dis Child* 1990;65:1199-1202.

Stanhope R, et al. *Acta Paediatr Scand* 1986;75:779.

Editor's comment: *I concur with the authors that the probability of a pathologic finding in a MRI study is high in multiple pituitary deficiencies, in diabetes insipidus, and in precocious puberty of very early onset. In most patients with hypogonadotropic hypogonadism, with or without anosmia, and in patients with precocious puberty occurring after 3 or 4 years of age, a functional etiology of the pathologic process is probable. It is unclear at this time whether all patients with isolated GHD should have an MRI. However, if an abnormality is detected by MRI in a patient with isolated GHD, the patient should be followed closely for the possible development of multiple pituitary hormone deficiencies.*

Jean-Claude Job, MD

Adult Panhypopituitarism Presenting as Idiopathic Growth Hormone Deficiency in Childhood

The goal of this short clinical report is to suggest that protracted follow-up is needed after treatment of growth hormone deficiency (GHD), and that reassessment of pituitary function in adult life may be useful.

The first patient reported was a girl whose GHD was diagnosed at age 11 years. At 11 years, no other pituitary functions were involved, and the CT scan of the pituitary was considered normal. She was treated with GH for 7 years and grew rapidly. However, at 19 years, she had thyrotropin, corticotropin, and follicle-stimulating hormone releasing hormone deficiencies. A repeat CT scan showed an empty sella with a thin pituitary stalk and no glandular tissue.

The second patient was a boy, referred at 9 years of age with a height deficiency of -3.6 standard deviations (SD). Like in the first patient, no deficiency other than that of GH was evident. The pituitary seemed normal on a CT scan. He was then treated with GH for 10 years. Hypothyroidism was discovered at age 16 years. Corticotropin and gonadotropin deficiency became obvious at 18 years. A repeat CT scan showed a small sella containing apparently normal pituitary tissue.

The authors discuss the possibility of progressive loss of anterior pituitary function, irrespective of a radiologically demonstrable lesion. And they stress the importance of continuous attention to patients with so-called isolated GHD.

Crowne EC, et al. *Acta Paediatr Scand* 1991;80:255-258.

Editor's comment: *This article augments those cases reported in "Review: Magnetic Resonance Imaging in Patients With Hypothalamic and/or Pituitary Disorders," which is in this issue of* GGH (see page 9). *Evidence is rapidly accumulating that a diagnosis of isolated GHD today does not mean that deficiencies of other tropic hormones will not appear subsequently even if initial CT scans reveal no pathology.*

Jean-Claude Job, MD

A New Syndrome of Congenital Hypoparathyroidism, Severe Growth Failure, and Dysmorphic Features

Sanjad et al describe the identification of 12 patients with an unusual syndrome of congenital hypoparathyroidism associated with growth failure and dysmorphic features distinctive from those of the DiGeorge syndrome. The onset of symptoms of hypocalcemia occurred between 1 and 30 days of life. Ten of the 12 patients had parents who were first cousins. All but one patient had severe intrauterine growth retardation, with birth weights ranging from 1,500 to 2,150 g. All had moderate to severe hypocalcemia and hyperphosphatemia but no chromosomal abnormalities detectable by karyotyping. Physical features of this syndrome include: deep set eyes, microcephaly, thin lips, beak nose tip, external ear anomalies, micrognathia, depressed nasal bridge (Figure 1). Mental retardation was found in all patients and all had severe postnatal growth retardation despite treatment with vitamin D and calcium supplements, which normalized serum calcium levels.

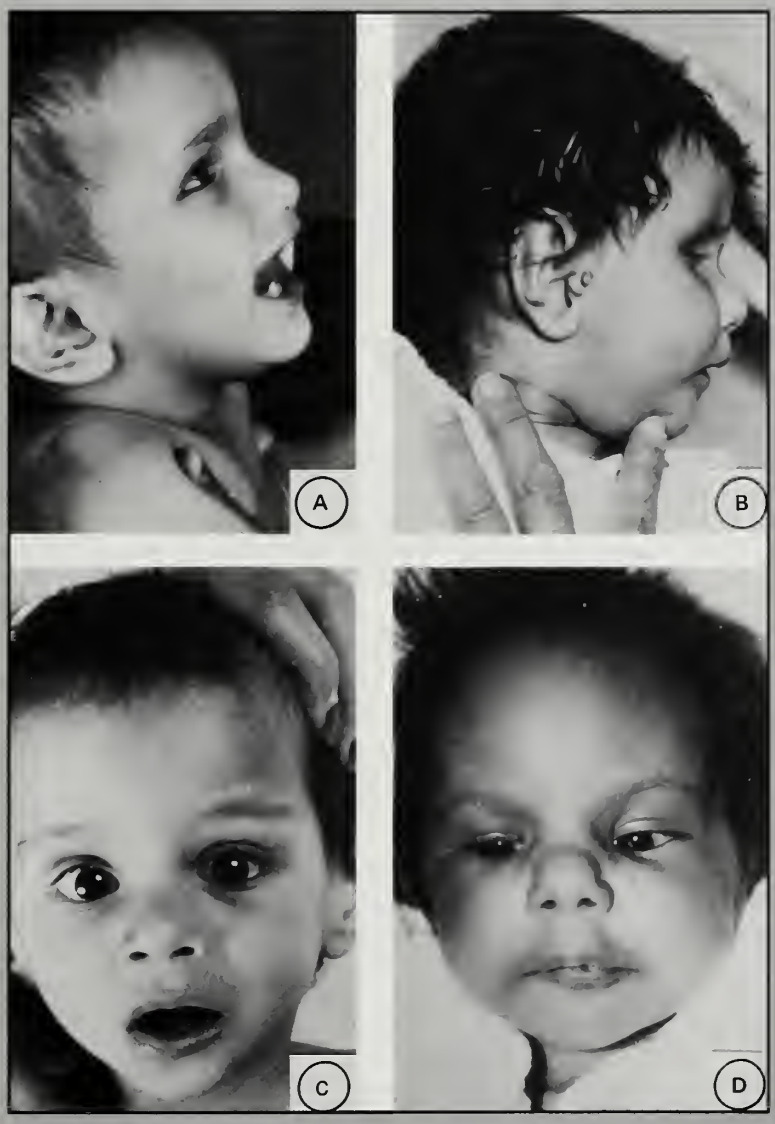

Figure 1: (A) Case 4: girl with deep set eyes, micrognathia, thin lips, and simple malformed posteriorly rotated ears. (B) Case 2: girl with prominent forehead, depressed nasal bridge, deep set eyes, micrognathia, thin lips, and preauricular tags. (C) Case 1: boy with deep set eyes, broad nasal bridge, prominent forehead, and micrognathia. (D) Case 5: boy with deep set eyes, epicanthic folds, broad nasal bridge.

Sanjad SA, et al. *Arch Dis Child* 1991;66:193-196.

Editor's comments: *The authors indicate that they were unable to determine the pathophysiology of the hypoparathyroidism in these individuals. They point out that since no patient showed clinical evidence of T-cell deficiency, it is unlikely that these patients represent a variant of DiGeorge syndrome. In addition, this syndrome is the only syndrome with hypoparathyroidism in association with intrauterine growth retardation. Although all of the patients to date have been identified in Saudi Arabia, it is important for pediatric geneticists and endocrinologists to be on the lookout for further cases of this interesting syndrome.*

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Decreased Growth Velocity Before Insulin-Dependent Diabetes Mellitus

Growth was studied prospectively in 12 nondiabetic identical twins <14 years, and in their cotwins with insulin-dependent diabetes mellitus (IDDM) to determine whether changes in growth occur before the onset of IDDM. Seven of the 12 nondiabetic twins subsequently developed IDDM. A significantly reduced growth velocity (GV) was observed in these 7 as compared with their diabetic cotwins and as compared with those who did not develop IDDM. The nadir of growth in the twins who developed diabetes occurred a mean of 1.2 years before diagnosis (range, 0.3 to 2.3 years). Islet cell antibodies were observed in all 7 of the prediabetic twins in contrast to 0 of 5 of those who did not develop IDDM. In 4 prediabetic twins the decreased growth preceded impaired glucose tolerance. The prediabetic twins had lower testosterone (males) and estradiol (females) levels at the time of slow growth than did the diabetic, normally growing twins.

David R, et al. *Diabetes* 1991;40:211.

Editor's comment: *This is the first prospective study of growth prior to the onset of diabetes in monozygotic twins. As such, it is an exceedingly important study. Previous studies of growth before the onset of diabetes were retrospective and reported conflicting findings that children with diabetes were shorter, taller, or the same height as normals. The present study demonstrates that nondiabetic twins who will develop diabetes have a significant tendency for growth retardation (GV less than the 3rd percentile), as compared with their diabetic twins. In addition, there is some evidence to suggest that testosterone and estradiol may be lower in the prediabetic individuals as compared with their diabetic twins. The etiology of the decreased GV in relation to these findings, however, remains unclear. Regardless, there must be subtle metabolic changes that require more detailed studies of GH and gonadotropin secretion. From these studies one can presume that many children with identified islet cell antibodies will become diabetic and GV will decrease to abnormally low values (less than the 3rd percentile growth velocity for age) before any evidence of diabetes is present.*

William L. Clarke, MD

Estrogen Treatment of Tall Girls: Dose Dependency of Effects on Subsequent Growth and IGF-1 Levels in Blood

This retrospective study compares the effects of ethinyl estradiol 250, 500, or 1,000 μg/d given 3 weeks of each 4-week cycle in very tall girls. Each study consisted of 15 to 21 girls who were in the same age range (13.5 ± 1.1 years), with similar bone ages (12.6 ± 0.9 years), similar height predictions (186.2 ± 3.1 cm), and duration of treatment (1.9 ± 0.6 years). Most received a progesterone analogue during the third week of each cycle.

In the 3 groups, the difference between final and predicted height was similar: 5.5 ± 2.7, 5.9 ± 3.3, and 5.6 ± 2.7 years. Follow-up of plasma insulin-like growth factor 1 (IGF-1) levels revealed a significant decrease with 500 or 1,000 μg/d of ethinyl estradiol but not with 250 μg/d.

The authors conclude that a dose of 250 μg of ethinyl estradiol per day for 3 of every 4 weeks is as potent in reducing final height in tall girls as higher doses.

Savan H, et al. *Acta Paediatr Scand* 1991;80:328-332.

Editor's comment: *The use of large doses of estrogen to reduce stature in tall girls was initiated by Wettenhall in 1955 (*J Pediatr *1975;86:602). He used diethylstilbestrol with limited success in reducing stature (an average of -3.5 cm), as the girls he treated had epiphyses that were nearly fused to the metaphyses when treatment was initiated (mean bone age, 13.2 years). The value of his study was that 87 girls were followed for >15 years. The only toxicity reported was the development of paraovarian cysts in 2 girls and superficial thrombophlebitis in another.*

*Bierich (*Pediatrics *1978;62:1196 and* Gynakologe *1983;16:72) reported his experience with 41 girls whose predicted heights were >180 cm. Conjugated estrogen (7.5 mg/d) was used (plus 7 days of progesterone each 28 days). Fifty percent of the girls were menstruating when treatment started. Because skeletal maturation advanced 3.7 times faster than body height, growth retardation as compared with predicted height was -8.3 ± 2.1 cm in the premenarcheal girls and -6.8 ± 1.6 cm in the menarcheal girls. Doses of 0.3 to 0.5 mg/d of ethinyl estradiol produced similar results. No side*

*effects were observed in relation to high-dose treatment including no increases in triglycerides and cholesterol. However, Weninger et al subsequently reported (*Acta Paediatr Scand *1987;76:500) hyperlipidemia in association with ethinyl estradiol therapy at this dose level.*

*An important question is: Could a smaller dose of ethinyl estradiol be as effective as the usually recommended larger dose? Bartsch et al (*Eur J Ped *1988; 147:59) evaluated the use of 0.1 mg/d for 2 years in 25 tall girls. They reported that the reduction in predicted adult height achieved by estrogen treatment averaged 7.4 cm in girls whose bone ages were >12.5 years. They concluded that the higher dosages offer little advantage. Gruters et al (*Eur J Ped *1989;149:11) studied 2 comparable groups of tall girls: the first group receiving 0.3 - 0.5 mg/d ethinyl estradiol, and the second 0.1 mg/d ethinyl estradiol. The different doses had similar effects on final height reduction.*

The current abstract compares several intermediate doses (0.25, 0.50, and 1.0 mg/d) and concludes that the effects are comparable. The consensus seems to be that doses prescribed to treat tall girls over the past 20 years may be unnecessarily high. A response from the readers through the "Letters to the Editor" column is welcome.

Jean-Claude Job, MD

Growth Hormone Gene Deficiency: Hot Spots for Growth Hormone Gene Deletions in Homologous Regions Outside of Alu Repeats

Most types of growth hormone deficiency (GHD) do not involve the growth hormone gene, but there is a rare familial type of GHD, type 1A, that is caused by deletion of the growth hormone N gene on each chromosome 17 in affected individuals. The authors examined the specific mutation in 10 patients with type 1A GHD. These patients represented different geographic origins. Different size deletions were found in each family. The deletions appear to be related to abnormal pairing in the areas that flank the growth hormone gene, which leads to abnormal cross-overs. These areas have many Alu repeats. Since these are areas of recombination, it is possible to mismatch if there are different numbers of Alu repeats on the chromosomes inherited from mother and father. The mismatch of Alu repeats appears to make for hot spots of abnormal recombination. The areas of Alu repeats are clearly important areas for producing deletions that result in GHD since all the patients studied have this kind of mismatch deletion.

Vnencak-Jones CL, et al. *Science* 1990;450:1745-1748.

Editor's comment: *Type 1A GHD is quite rare and presents a problem to the clinician in that when growth hormone is given, antibodies to it usually develop. Nevertheless, the study of these families has led to a better understanding of how mutations occur, at least in the case of the growth hormone gene. It appears that they are likely to occur because of mismatching of the chromosome areas outside the gene. The study is particularly important for understanding what leads to mutations of this type.*

Judith G. Hall, MD

Adult Height in Boys and Girls With Untreated Short Stature and Constitutional Delay of Growth and Puberty: Accuracy of 5 Different Methods

The height predictions of 5 methods were compared with ultimate adult height in 37 boys and 32 girls with short stature associated with constitutional delay of growth and puberty (CDGP). The boys were seen initially at a chronologic age (CA) of 14.8 ± 1.7 years and the girls at 12.9 ± 2.6 years. The groups were seen ultimately at 23.1 years and 21.1 years.

For boys, the adult height was overestimated by calculation of the target height, as compared with the ultimate height, by 1.7 ± 5.7 cm. The overestimate for girls by the target height method was 0.65 ± 4.31 cm. The Roche-Wainer-Thissen (RWT) method was the most accurate predictor for boys, underestimating the adult height by 0.53 ± 4.37 cm. In girls, the RWT method was less accurate as it overestimated the adult height by 2.6 ± 3.2 cm. The Bayley-Pinneau (BP) method overestimated significantly the ultimate height for boys (3.1 ± 5.5 cm) and underestimated the height for girls (0.8 ± 3.6 cm). The TW2 method underestimated significantly for both boys (1.76 ± 3.27 cm) and girls (4.17 ± 5.35 cm). The TW1 method was even less

predictable. In girls, all prediction methods gave similar results, with no method being significantly superior to the others. In boys, the RWT method offered the best estimates of adult height.

In the studies reported here in patients with CDGP, the adult height by the BP method was overestimated by 3.1 cm, which compares with the data in other series by this method (overestimations of 2 to 4 cm). The authors conclude that patients with CDGP usually reach an adult height in the lower normal range. The adult height of patients with CDGP is below the target height and does not reach the height standard deviation score (SDS) for bone age observed at the initial visit.

Bramswig JH, et al. *J Pediatr* 1990;117:886-891.

Editor's comment: *The observed discrepancies of overestimations and underestimations of each method may relate to the particular ethnic group evaluated; thus, obtaining data for a particular center and the ethnic group(s) served by that center may be important in determining accurate predictions. Nevertheless, this is an important paper that demonstrates the variability of 5 different methods of height prediction within 1 clinic population for boys and girls with CDGP. These data do not necessarily apply to children who do not have CDGP. Particularly impressive in the data presented are the large measurements related to SDS. It is important for both clinicians and investigators who use height predictions to evaluate growth promoting therapy to know the tendency of each method to underestimate or overestimate adult height.*

Judith G. Hall, MD

Growth Acceleration and Final Height After Treatment for Delayed Diagnosis of Celiac Disease

Short stature and growth failure may be the only clinical presentation of the so-called occult form of celiac disease (CD). This paper reports on 24 patients over 4 years of age in whom CD was diagnosed. Their initial presentation was short stature or retarded growth, with heights below the 5th percentile but without any other overt symptoms. The effect of treatment with a gluten-free diet (GFD) on catch-up growth and final height was determined.

Small-bowel biopsy demonstrated mucosal atrophy in all 24 patients. Antigluten antibody (AGA) titers were also found to be abnormal in the 13 of 24 patients whose levels were measured. Weight and height velocities, pubertal staging, bone age (BA), target height (TH, based on midparental height), and predicted height (PH, according to Tanner) were recorded at diagnosis and periodically after treatment was begun with GFD.

At diagnosis, 82% of patients were below the 3rd percentile for height and 58% were below the 3rd percentile for weight. Nearly all of the patients (95%) had a delayed BA compared with chronologic age (range of delay, 1 to 6 years). All patients had catch-up growth following the institution of GFD, with increased height and weight velocities rapidly achieved during the first year of GFD. After 1 year of treatment, 87% of patients had a stable height velocity above the 50th percentile and their height standard deviation score (HSDS) improved significantly. By the third year, their HSDS showed less stature reduction than that observed at the time of diagnosis (-1.77 vs -2.52). The patients who reached an appropriate TH for midparental height were those in whom the diagnosis was made and treatment was started before puberty. In contrast, the patients who did not achieve a satisfactory final adult height were those who began dietary treatment after the onset of puberty.

Bosio L, et al. *J Pediatr Gastroenterol Nutr* 1990;11:324-329.

Editor's comment: *Although the paper by Bosio et al does not address all pertinent issues of CD diagnosis and prognosis, it adds to the large volume of reports indicating that relatively asymptomatic short-statured children without weight deficits for height but with various degrees of retarded BA may have CD as the cause of their poor growth. This paper also confirms that the diagnosis of CD can be established only by a small-bowel biopsy, which shows the typical histologic findings, whereas other measurements of intestinal function (ie, xylose tolerance) may fail to detect any abnormality.*

The paper by Bosio et al also suggests that when an appropriate diagnosis is made and when timely dietary treatment is given these patients exhibit catch-up growth and attain an appropriate height based on midparental height. In contrast, when there is a delay in the diagnosis and treatment with GFD is initiated after the onset of puberty, there may be an unsatisfactory final adult height.

Therefore, the clinician must be alert and must consider CD as a cause of short stature. Of course, there are other, perhaps more important reasons besides stature mandating that the accurate diagnosis of CD be made as early as possible. Since the only way to rule out CD is by small-bowel biopsy, the clinician must keep in mind the clinical indications for this procedure when a short child is evaluated. These vary in accordance with the geographic location and with the clinical history of the patient. In areas of the world where CD is frequent, it should be high on the list in the differential diagnosis of short stature. The clinical history usually reveals clues for consideration of CD. This entity is not "occult" in the majority of these subjects. In the paper by Bosio et al summarized above as well as in other publications on the subject, it is clear that these patients have a frequent history of diarrhea, poor weight gain, and other gastrointestinal symptoms in infancy. These symptoms are often considered not important enough to be thoroughly evaluated by the physician, although patients are often treated by dietary manipulations. Other important clues to alert the clinician include a deteriorating height and weight pattern of growth and the presence of nutritional deficits, ie, iron deficiency associated with short stature. The paper by Bosio et al provided no data on the growth patterns that preceded the diagnosis of CD in their patients, nor did it contain information regarding the presence or absence of nutritional abnormalities like iron deficiency. Patients with CD usually have a growth pattern typical of nutritional dwarfing with decelerating weight gain and height velocity, although they may not have weight loss or body weight deficits for height. Also, they often exhibit iron deficiency even though there may be no anemia. It is clear that following an appropriate diagnosis and treatment with GFD, the patient will exhibit catch-up growth and weight gain, which may occur rapidly during the initial stages of treatment.

Fima Lifshitz, MD

Review: Mapping for the Marfan Syndrome Gene

Marfan syndrome is a relatively common inherited connective tissue disorder characterized by tall stature, dislocated lens, and cardiovascular abnormalities including aortic dilatation and dissection. Despite intensive research carried out in various laboratories over the years, nothing has been known about the genetic defect leading to the syndrome. Linkage analyses have excluded many of the suspected genes. Recently Kainulainen et al, using linkage analyses with polymorphic markers of the human genome, mapped the genetic defect to the long arm of chromosome 15 in 5 families with Marfan syndrome. This methodology now serves as a diagnostic test in families in which consegregation of these markers with the disease has been confirmed.

The basic defect now appears to be in the microfibrillar system, which is widely distributed in the extracellular space. Hollister et al demonstrated that there is a striking lack of fibrillin in both skin sections and dermal fibroblasts from Marfan patients as compared with normal subjects. However, there are several genes in the identified affected area of chromosome 17, including those for chondroitin sulfate, protroglycan I core protein, cardiac muscle alpha-actin, and type I collagen receptor, that could be implicated. The specific gene remains to be isolated.

Kainulainen K, et al. *N Engl J Med* 1990;323:935-939.

Hollister DW, et al. *N Engl J Med* 1990;323:152-159.

Editorial in *Lancet* 1990;336:973.

Judith G. Hall, MD

Iodine and Selenium Deficiency Associated With Cretinism in Northern Zaire

It is known that endemic cretinism is associated with severe iodine deficiency, but the reason for the variable geographic distribution of its myxedematous form is not clear. This study examined the selenium status of persons living in the endemic goiter belt of northern Zaire, where myxedematous cretinism, characterized by goiter, overt hypothyroidism, and stunted growth, is predominant.

The study was conducted in 2 rural villages in the core of the endemic goiter area and included 52 normal schoolchildren (aged 9 to 18 years) and 28 cretins (aged 3 to 25 years). Reference values

were obtained from adults hospitalized for medical checkups (n=30) in another, less severely iodine-deficient area and from volunteer healthy medical workers (n=9) in an iodine-nondeficient location. Stature and the presence of visible goiter were recorded. Blood was drawn for baseline serum thyroid indexes, serum and red blood cell (RBC) selenium, and erythrocyte glutathione peroxidase, glucose-6-phosphate dehydrogenase, glutathione reductase, pyruvate kinase, and hemoglobin A, A_2, and S. Urine for iodide was also analyzed. The schoolchildren and cretins in 1 village were supplemented orally for 2 months with 50 μg selenium/d while the participants from the second village received a placebo. All subjects in both villages were subdivided for the next 4 months into a supplemented group (100 μg selenium/d) and a placebo group.

The schoolchildren often presented with visible goiter (41 out of 53) and had biochemical evidence of hypothyroidism (low thyroxine [T_4] and triiodothyronine [T_3] and high thyrotropin [TSH]) but the cretins showed biologic signs of severe hypothyroidism (very stunted growth, low T_4, and markedly elevated TSH). Seven of 30 of the hospitalized adults had goiters and all were euthyroid, whereas none of the volunteers had any evidence of thyroid dysfunction.

The serum selenium of school children and cretins (343 ± 176 nmol/L vs 443 ± 188 nmol/L, $P>0.1$) was markedly lower than that of the adult patients (753 ± 355 nmol/L) or medical workers (2,555 ± 347 nmol/L). The erythrocyte glutathione peroxidase concentrations followed the same pattern as the selenium. However, the other erythrocyte enzyme activity (pyruvate kinase, glucose-6-phosphate dehydrogenase, pyruvate kinase, and glutathione reductase) and the prevalence of abnormal hemoglobin (consistent with ß-thalassemia trait) were similar in all groups. The urine iodine was very low in school children and cretins (0.20 and 0.16 μmol/L) and was moderately decreased in adult patients (0.37), but was high in the medical workers (4.57).

Multifactorial analysis was significant for the effect of iodine on thyroid hormones. The effect of serum selenium was not significant. A supplementary effect of erythrocyte glutathione reductase was also significant for TSH, T_4, and free T_4 levels in multiple regression analysis. After 2 months of selenium supplementation, the serum selenium concentrations became normal, whereas the erythrocyte glutathione peroxidase continued to increase and reached normal concentrations only after 6 months of treatment. The increase in serum selenium and erythrocyte glutathione peroxidase after 2 months of supplementation was more pronounced in the cretins than in the schoolchildren but was not significantly different after 4 to 6 months.

The authors conclude that there is a severe selenium deficiency in the core of the northern Zaire goiter belt, which reinforces the hypothesis of and association with endemic myxedematous cretinism.

Vanderpas JP, et al. *Am J Clin Nutr* 1990;52:1087-1093.

Editor's comment: *These data clearly document the presence of selenium deficiency associated with iodine-deficient goiter and cretinism in Zaire. In China, selenium deficiency has been reported to produce a cardiomyopathy called Keshan disease and osteoarthropathy called Kashin-Beck disease. However, the selenium deficiency observed in these 2 conditions may be more severe than the condition documented in association with iodine deficiency, goiter, and cretinism since there was no cardiomyopathy or osteoarthropathy detected in the Zaire study. Macrocytosis, lightening of hair and skin color, and abnormalities of liver enzymes have also been noted in patients with selenium deficiency given total parenteral nutrition for long periods. These findings were not addressed in the study of Vanderpas et al in Zaire.*

In other areas of the world it has also been noted that where iodine-deficiency goiter is prevalent, the presence of cretinism is more frequent when there is also an overlap with selenium deficiency. The converse is also true; in selenium-deficient areas where there is a high supply of iodine there is no increased rate of cretinism or thyroid function abnormalities even when there is endemic goiter.

The possibility of an interaction of combined deficiencies of iodine and selenium leading to derangements of thyroid function should be considered. There are data indicating that hydrogen peroxide generated at the apical membranes of thyroid cells is necessary to oxidize thyrosal residues of thyroglobulin in the formation of thyroid hormones. Thus, the synthesis of excess hydrogen peroxide in a stimulated gland and the lack of hydrogen peroxide-detoxifying enzyme would progressively induce more severe thyroid hormone deficiencies. Additionally, selenium may also play a role in thyroid function by modulating the iodinases necessary for conversion of T_4 into T_3 and reverse T_3 (rT_3) in extrathyroid tissues.

Further studies remain to be done to ascertain the clinical and public health benefits of selenium supplementation in areas of the world where there is selenium deficiency, as well as in other types of goiters more commonly observed in our population.

Fima Lifshitz, MD

Nitrogen Kinetics and Growth in Short Children Treated With Growth Hormone

Dempsher et al studied the acute effects of growth hormone (GH) on retention of ^{15}N-labeled amino acids and its relationship to the response to GH treatment in 37 short children.

The patients were recruited from the Washington University Pediatric Endocrinology Clinic. They were prepubertal, between 6 and 14 years of age, and had heights more than 2 standard deviations (SD) below the mean for age. Patients with chronic medical illnesses were excluded, as were girls with chromosome abnormalities. The group as a whole had a mean bone age (BA) delay of slightly more than 2 years and biologic parents with mean heights below the 50th percentile for normal adults. Pretreatment height velocity measurements were recorded for a minimum of 1 year and averaged 4.7 ± 1.2 cm/yr.

The patients underwent the following studies:

1. GH secretion: GH response was measured by radioimmunoassay (RIA) after clonidine and insulin stimulation. Thirty-four of the 37 children had normal GH levels as measured by provocative stimuli; 3 children had values below 7 ng/mL in response to both tests. These 3 patients were classified as GH deficient (GHD). However, because the subsequent response of this group of 3 children to acute and chronic GH supplementation was indistinguishable from those of the remaining 34 subjects, their results were included with the rest of the study group.
2. GH molecule: In addition to the GH measurement by conventional polyclonal RIA, the highest GH concentrations in the 2 plasma samples from each provocative test were remeasured by monoclonal IRMA or by IM-9 or human liver radioreceptor assays. All of the 34 non-GHD subjects had a normal GH molecule as estimated from the ratio of IRMA radioreceptor to RIA.
3. Growth hormone-binding protein (GHBP): None of the 37 subjects had GHBP alterations.
4. GH and insulin-like growth factor 1 (IGF-1) genes: The GH gene was analyzed in all 37 subjects and in 10 controls of normal stature. Normal patterns were observed in all subjects with each of the 3 restriction enzymes used. Additionally, the IGF-1 gene was studied in these subjects. Analysis of the chromosomal DNA failed to reveal any abnormal mutation.
5. Fibroblast responsiveness to IGF-1: A punch biopsy skin specimen was obtained in 34 of the 37 children. Cultured fibroblasts were assessed for aminobutyric acid uptake response to recombinant human IGF-1. All the cell lines responded normally.
6. Nitrogen kinetic response: Each child was admitted to the Clinical Research Unit for 9 days to study the acute effects of GH administration on nitrogen kinetics. The patients consumed an isocaloric diet containing 1 g of protein per kilogram daily for the week before the admission, and continued with the same intake during the 9 days of hospitalization. On the second and sixth hospital days, a dose of mixed ^{15}N-labeled amino acids (1 mg ^{15}N/kg body weight) was given orally with breakfast. The mixture contained leucine, valine, methionine, phenylalanine, lysine, alanine, aspartic acid, glutamic acid, glycine, serine, and tryosine. From the morning of the fifth day until discharge, recombinant human GH (rhGH) was injected sc every 12 hours at a dose of 16 µg/kg body weight.

   Daily total urinary nitrogen excretion declined slightly but significantly ($P<0.05$) between the first and second day of hospitalization but remained unchanged from the second to the fourth day. GH injections, begun on the fifth hospital day, produced a second decline in total urinary nitrogen, which achieved a new constant level on days 6 through 8. The total cumulative excretion of ^{15}N before the administration of GH averaged 20.3% of the dose. During acute supplementation with GH, ^{15}N excretion declined an average of 31 ± 10%, but the change varied considerably among subjects. The ^{15}N excretion of the 3 GHD children diminished by 31.5, 31.8, and 50.5%. Statistical analysis failed to demonstrate a significant relationship among the subjects' clinical characteristics, BA delay, IGF-1 level, and the degree of nitrogen retention after GH administration.

   Whole body protein turnover, synthesis, and catabolism were also evaluated. GH challenge increased the net body protein accretion (synthesis minus catabolism) by more than 200%, from 0.14 ± 0.30 to 0.35 ± 0.02 g/kg^{-1} day^{-1} ($P<0.001$).
7. Changes in other parameters in response to acute rhGH administration: IGF-1 levels rose significantly with GH administration, but this increment did not correlate significantly with any of the protein kinetic indexes studied.

   The acute challenge with rhGH caused no change in circulating GHBP, nor were

any significant changes observed in plasma glucose or insulin values. However, daily urinary C peptide excretion increased significantly ($P<0.05$) within 24 hours of GH administration and remained elevated throughout the period of GH treatment. Serum osteocalcin levels remained unchanged after 4 days of GH injections.

8. Growth response: On discharge from the hospital, each patient was treated with rhGH at the dose of 75 ug/kg body weight sc tiw. The children were assessed every 3 months over the next 6 to 12 months. Of the 37 subjects who had completed the initial studies, 2 discontinued follow-up. In another 3 children, GH therapy was discontinued at 6 months because height velocities had not increased by more than 2 cm/yr above pretreatment values. The final examination performed in the remaining "responders" at the 12-month visit showed a mean increase in height velocity from 4.7 ± 1.2 to 8.3 ± 1.6 cm/yr ($P<0.001$), producing significant changes in the height Z score and the height velocity Z score for chronologic age (CA) and BA. The 3 GHD children increased their absolute height velocities by 2.6, 6.3, and 9.2 cm/yr.
9. Predictors of response to GH treatment: Neither the pretreatment IGF-1 nor the acute change IGF-1 to GH challenge predicted the long-term growth response. Only a low pretreatment height velocity correlated significantly with the change in growth rate after 1 year of treatment ($r = -0.6$, $P<0.001$).

Neither the measured growth rates nor the increments above pretreatment values at 3, 6, or 12 months of therapy correlated with any of the indices of protein dynamics. However, when the change in height velocity measured after 1 year of treatment was expressed as Z score, there was a weak but significant correlation ($r = 0.37$, $P = 0.03$) with the change in ^{15}N retention to the acute GH challenge. This relationship was considered by the authors as too weak to be used as a predictor of GH response in individual cases.

Dempsher DP, et al. *Pediatr Res* 1990;28:394-400.

Editor's comment: *This study is a very comprehensive and sophisticated one. It attempted to ascertain a functional test that would predict the long-term efficacy of GH treatment in short children. The authors measured many of the possible alterations that could lead to disturbed growth and/or short stature in children. They found that in the great majority of instances there was no GHD, nor were there abnormalities in the GH molecule, GHBP, response to IGF, or in the nitrogen kinetic response to GH administration. Yet these children were short.*

By sophisticated analysis with stable isotope ^{15}N-labeled amino acids, total body nitrogen retention as well as protein synthesis, breakdown, and net anabolism were shown to increase with the acute administration of GH. However, the degree of positivity of the nitrogen balance enhanced by GH was extremely variable. Also, the levels of nonabsorption found among short children who had no GH deficits were similar to those who exhibited classic GHD. Moreover, none of the above mentioned measurements were of value in predicting the long-term effects of rhGH in inducing enhanced growth responses.

Unfortunately, there were no measurements of spontaneous GH secretory rates in these children. Perhaps the spontaneous GH secretion may have shed some light on the variability of response to GH administration. It is possible to speculate that those who had adequate spontaneous GH secretion had the weakest anabolic responses, while those with inappropriate spontaneous secretion may have had the strongest responses.

Once again, there is strong confirmation in this paper of the great value of careful clinical observation and close monitoring of long-term growth in children. The only variable that was of value in predicting the response to GH was the children's pretreatment growth rate. Those who grew at the lowest rate had the most significant enhancement of growth with GH therapy, whereas those who had appropriate growth before treatment had the least significant responses. Thus, the clinician can be thoroughly assured that sophisticated laboratory measurements are no substitute for careful measurements of growth both in assessing short children and in determining the need for therapeutic trials of rhGH. Additionally, caution is advised regarding the questionable improvement of predicted adult height, even among those patients who had significant improvement in growth velocity during the first year of GH treatment.

Fima Lifshitz, MD

Erratum

In *GROWTH, Genetics, & Hormones* Vol. 7, No. 2 (June 1991), an error on page 2, Table1 incorrectly shows the nomenclature for DRßIII italicized and it should not be italicized. DRßIII is not a pseudogene but is expressed.

Serum Bone GLa Protein (BGP): A Potential Marker of Growth Hormone Deficiency and the Response to Growth Hormone Therapy

Serum bone GLa protein (BGP), a calcium-binding protein of the bone matrix, is the most important noncollagenous protein in the skeleton. BGP has been shown to be an important indicator of the rate of bone formation. In this paper, Johansen and coinvestigators studied the usefulness of BGP in predicting the long-term reponse of growth hormone (GH)-deficient patients to GH treatment.

Sixty-six GH-deficient children aged 6 to 18 years, 49 boys and 17 girls, were studied before and after 3, 6, 9, and 12 months of daily GH treatment. Patients were divided into 2 groups: those who remained prepubertal throughout the study period were included in group 1 (n = 51 ; mean age, 11.2 years; 35 boys and 16 girls), while those who had entered stage 3 of puberty before starting treatment or those who reached that stage during the study were included in group 2 (n = 15; mean age, 14.2 years; 14 boys and 1 girl). Serum BGP concentrations were determined by radioimmunoassay (RIA). Height velocities were estimated from all available height measurements. The change in height velocity from 0 to 12 months of treatment was correlated with the change in BGP concentration at each period.

The mean pretreatment height velocities were 4.5 and 5.1 cm/yr in groups 1 and 2, respectively, while the mean height velocities at 12 months of GH treatment were 8.3 and 8.2 cm/yr in groups 1 and 2, respectively. The mean BGP in patients before treatment was significantly lower than the levels found by other investigators in normal controls ($P < 0.001$ in prepubertal patients and $P < 0.01$ in pubertal patients). The BGP levels increased significantly in both groups of GH-deficient children, reaching normal levels at 3 months, and plateaued at a higher level thereafter.

The authors conclude that the present study demonstrates that determination of serum BGP is a valid contribution to the prediction of growth response after 12 months of treatment; the change in serum BGP determined after 3 months of therapy was able to predict the height at 12 months of therapy with the same validity as the prediction at 6 months without using BGP. The present study suggests that determination of serum BGP may help to assess the extent to which bone metabolism is affected in GH-deficient children. Furthermore, serum BGP could be particularly useful to monitor treatment. Measurement of changes in serum BGP after short-term GH administration may thus help to identify those children who will benefit from long-term therapy as well as those who will not respond to therapy.

Johansen JS, et al. *J Clin Endocrinol Metab* 1990;71:122-126.

Editor's comment: *The efficacy of GH treatment in improving the ultimate height of a child is an important question that has been difficult to address. The response to GH has been widely variable even among patients with GH deficiency. For example, in this study with supposedly GH-deficient subjects there was a normal growth rate before treatment and an improved growth velocity of +3 cm/yr with GH treatment. The growth of these patients and the response to GH is more like that of normal short-statured patients. Usually, GH-deficient patients grow less than 4 cm/yr and exhibit catch-up growth when treated with human GH.*

However, it has always been difficult to predict who will benefit most from long-term GH treatment. The good predictive value of serum BGP concentrations reported here at 3 months of treatment, if duplicated by other studies, might help the clinician decide which patient will benefit most from treatment. Potentially this would facilitate optimization of dosing regimen, growth response, and monetary expenditures. For example, if the dose of GH being employed does not increase the BGP in an ordinary patient, it may be beneficial to increase the dose; or, if the patient is being treated 3 times per week, it may be better to give it daily. The prompt recognition of poor GH response before wasting many months of treatment would be of great benefit.

Other investigators have studied the usefulness of procollagen levels as a biochemical marker for growth.[1,2] Various methods are available to measure either type I (pColl-1-C) or type III procollagen (P III NP). More studies have been done on P III NP, which also reflects generalized somatic growth, presumably because this assay has been simplified and RIA kits are commercially available. P III NP concentrations have also been found to be good predictors of growth response to GH treatment.[3,4] Comparative studies between BGP and these other procollagen measurements have not been done. This approach is very important.

Fima Lifshitz, MD

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November 1-3, 1991 PG Symposium on Ped Diab & Endo (sponsored by the ISGD), New Delhi, India. Info: Prof. I.C. Verma, All India Institute of Medical Sciences, Genetics Unit, Dept of Pediatrics, Old Operation Theatre Building, Ansari Nagar, New Delhi 110 029, India.

January 3-11, 1992 Introduction to Endocrine Investigations, Pacific Grove, CA. Info: Ann Singer, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814. Fax: 301-571-1869.

May 4-8, 1992 Annual Meeting of the APS/SPR/APA, Baltimore Convention Center, Baltimore, MD. Abstract submission deadline 1/3/92. Info: APS/SPR/APA Program Office, 141 NW Point Blvd, PO Box 675, Elk Grove Village, IL 60009-0675. Fax: 708-427-1305

May 6-8, 1991 Annual Meeting of the LWPES, Baltimore, MD. Info: Dr. G.P. August, Secretary, LWPES, Children's National Medical Center, 111 Michigan Ave, Washington, DC 20010. Fax: 301-460-8846.

June 18-23, 1992 52nd Annual Meeting of the ADA, San Antonio, TX. Info: Meetings Dept, ADA, 1660 Duke St, Alexandria, VA 22314. Fax: 703-836-7439.

June 24-27, 1992 74th Annual Meeting of The Endocrine Society, San Antonio, TX. Info: Ann Singer, Meetings Manager, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814. Fax: 301-571-1869.

August 30 - September 5, 1992 9th Int'l Congress of Endocrinology, Nice, France. Info: NICE 92, c/o SOCF1, 14 Rue Mandar, 75002 Paris, France.

September 7-10, 1992 31st Annual Meeting of the ESPE, Zaragoza, Spain. Info: Dr. A. Ferrandez-Longas, Endocrine Unit, Miguel Servet Children's Hospital, Paseo Isabel la Catolica 3, 50009 Zaragoza, Spain. Tel: 34-976-355-700.

September 10-12, 1992 Int'l Congress on Growth Hormone and Somatomedins During Lifespan, Milan, Italy. Info: Drs. D. Cocchi and V. Locatelli, Dept of Pharmacology, School of Medicine, Univ of Milan, Via Vanvitelli, 32, 20129 Milan, Italy.

June 3-7, 1993 4th Joint Meeting of the ESPE/LWPES, San Francisco, CA. Info: Prof. M. Grumbach, Dept of Pediatrics, Univ of CA School of Medicine, San Francisco, CA 94143. Tel: 415-476-2244, Fax: 415-476-4009.

June 9-12, 1993 75th Annual Meeting of The Endocrine Society, Las Vegas, NV. Info: Ann Singer, Meetings Manager, The Endocrine Society, 9650 Rockville Pike, Bethesda, MD 20814. Fax: 301-571-1869.

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Rickets and Growth

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The relationship between growth and the skeletal manifestations of rickets is a time-honored clinical observation expressed in the classic dictum: "No growth, no rickets." Thus, in vitamin D-deficiency rickets the osseous manifestations depend on the age of onset and the relative growth rate of the different bones.[1] In the first year of life, the skull, upper limbs, and ribs are the fastest growing bones and thus prone to be affected. Accordingly, in the youngest infants craniotabes, frontal bossing, thickening of the wrist, and visible enlargement or palpable swelling of the costochondral junction (rachitic rosary or beads) are the characteristic skeletal manifestations. In the second year of life, the legs grow faster and the effect of weight bearing results in bowing of the legs, or genu varum. The angulation is especially pronounced at the junction of the lower third and upper two thirds of the leg. Later in childhood, vitamin D-deficiency rickets is rare but might occur during the growth spurt of puberty. The most prominent osseous manifestation of adolescent rickets is the occurrence of "knock knee," or genu valgum.

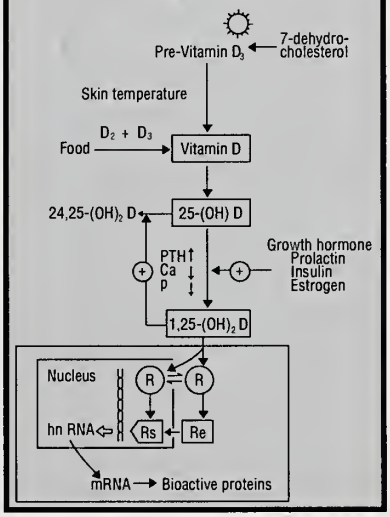
The assessment of the direct effects of vitamin D deficiency on growth is complicated by the degree and duration of bone deformities and the effects of concomitant nutritional deficiencies other than vitamin D. However, the general consensus among the pioneers in pediatrics at the turn of the century, when rickets was endemic in Northern Europe and North America, was that only part of the reduced height in patients with rickets could be ascribed to the deformity of the lower extremities.

VITAMIN D METABOLISM

Vitamin D was discovered in 1932. For many years, the vitamin was considered to be the active agent in controlling calcium and phosphate metabolism. The discovery of 25-hydroxyvitamin D (25-[OH]D) in 1968 paved the way for new progress, culminating with the identification of 1,25-dihydroxyvitamin D (1,25-$[OH]_2$D) in 1971. The appreciation of 1,25-$(OH)_2$D as the principal and most potent form of vitamin D, along with the elucidation of the molecular mechanism of action, which is analogous to that of classic steroid hormones, led to the recognition of vitamin D as a precursor of a potent steroid hormone rather than as a vitamin in the context of an essential nutritional substance. Recently, the identification of many new receptor-positive target tissues for 1,25-$(OH)_2$D beyond the classic ones of the intestine, the skeletal system, and the kidney implies that the hormone might have functions beyond its classic role in mineral homeostasis.[2]

The general term vitamin D refers to both vitamin D_2 (ergocalciferol), which originates in plants, and to vitamin D_3 (cholecalciferol), which is produced in the body. In humans, both compounds appear to be equipotent, and the requirements for the vitamin can be satisfied by either. Vitamin D undergoes 2 hydroxylation steps before it becomes biologically active (Figure 1). The first takes place in the liver to form 25-(OH)D,

Figure 1
Synthesis of 1,25-$(OH)_2$ Vitamin D



which is the major circulating metabolite of vitamin D. The serum concentration of 25-(OH)D reflects the vitamin D status of an individual, and is primarily determined by sunlight exposure and dietary supply of parent vitamin D. In temperate zones, the mean serum level is approximately 30 ng/mL (75 nmol/L), with a range of 10 to 50 ng/mL (25 to 125 nmol/L). Seasonal variation has to be taken into account, with the highest levels occurring in late summer and the lowest in late winter. A serum level

In This Issue

Letter to the Editors

Dear Drs. Blizzard, Stanhope, and Hindmarsh:

In your article on oxandrolone therapy (*GGH* 1991;Vol 3, No. 1:1-6) you gave significant discussion to studies supporting the use of oxandrolone to increase final adult height. However, you gave only the statement, "The effect of oxandrolone treatment on final adult height in Turner syndrome is somewhat controversial," in response to a significant body of literature suggesting oxandrolone makes no difference in adult height. In my study (*J Pediatr* 1984;104:365) of 66 adult patients with Turner syndrome, of whom 29 were treated with androgens (28 oxandrolone), no significant difference in adult height was noted. Moore previously reported some of these adult patients (whom you cite in defense of oxandrolone use) as successful results of oxandrolone therapy. In the end, these patients had no difference in ultimate height compared to untreated patients.

Others also found no significant difference in adult height (Lev-Ran; Mauri et al; Muritano and Job). They were similarly unimpressed with the effects of oxandrolone on final adult height.

The way in which the studies are described about patients with Turner syndrome in your article suggests that Stahnke et al found that oxandrolone therapy "increased final adult height in many patients." Stahnke did not present any data about final adult height in his study (in a letter to the Editor in the *Journal of Pediatrics,* 1980). He also stated that Joss and Zuppinger showed a significant increase in final adult height in all 15 oxandrolone-treated patients versus the untreated controls. This was true only for 8 patients who received 2 years of therapy. The difference between the control group and those who received only 1 year of therapy was not significant.

I agree that oxandrolone therapy increases growth velocity in Turner syndrome, but the evidence supporting its beneficial effect on adult height is limited and there is considerable evidence against this hypothesis. I also recognize that many feel that the "psychological

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below 8 ng/mL (20 nmol/L) indicates a state of vitamin D deficiency. Although 25-(OH)D is 2 to 5 times more potent than the parent hormone, it is not active at physiologic concentrations. To achieve full potency, 25-(OH)D is further hydroxylated in the kidney to 1,25-$(OH)_2$D. The circulating concentration is approximately one thousandth that of 25-(OH)D. In normal children, the serum level of 1,25-$(OH)_2$D ranges between 25 and 85 pg/mL (60 to 120 pmol/L). The higher values in infancy and adolescence probably reflect the need for increased intestinal calcium absorption during these periods of rapid growth.[3]

The conversion of 25-(OH)D to the active hormonal form 1,25-$(OH)_2$D is strictly regulated. The regulatory factors include parathyroid hormone (PTH), calcium, phosphate, and 1,25-$(OH)_2$D itself (Figure 1). PTH is the main stimulatory factor, and accordingly the serum level of 1,25-$(OH)_2$D is elevated in both primary and secondary hyperparathyroidism. The regulatory effect of calcium is probably indirect and mediated by stimulation of PTH secretion in hypocalcemic states. Dietary phosphate restriction and hypophosphatemia increase the serum concentration of 1,25-$(OH)_2$D, whereas high intake of phosphate decreases the level. Several endocrine factors have a direct or indirect effect on 1,25-$(OH)_2$D production (Figure 1). Children with growth hormone deficiency have normal serum concentrations of 1,25-$(OH)_2$D. High doses of growth hormone raise their serum levels of 1,25-$(OH)_2$D over the first week of treatment, whereas long-term replacement therapy does not cause such an effect.

Another major metabolite of 25-(OH)D formed in the kidney is 24,25-dihydroxyvitamin D (24,25-$[OH]_2$D). In normal children and adolescents, the serum concentration of 24,25-$(OH)_2$D is approximately 3% to 6% of the 25-(OH)D level. The production of 24,25-$(OH)_2$D is regulated by the same factors as the 1,25-$(OH)_2$D formation, but in the opposite direction (Figure 1). Although the physiologic role of 24,25-$(OH)_2$D remains unclear, it is regarded as an alternate path to the formation of 1,25-$(OH)_2$D to form a minimally potent instead of a maximally potent steroid.

BONE AND MINERAL METABOLISM

The importance of vitamin D for the maintenance of mineral homeostasis and normal bone growth and mineralization is apparent during states of either deficiency or resistance to vitamin D. Much of the effect of 1,25-$(OH)_2$D on bone mineralization is probably indirect, ie, providing minerals for incorporation into bone matrix through increased intestinal absorption of calcium. The essential bone lesion in rickets is an accumulated excess of osteoid tissue resulting from a lag in the mineralization of the cartilaginous epiphyseal plate.

The formation of new bone is a function of the osteoblasts, which possess 1,25-$(OH)_2$D receptors, and probably are the primary target cells for 1,25-$(OH)_2$D in bone. Receptor-mediated effects of the hormone include modulation of the proliferation of osteoblasts and the production of alkaline phosphatase and osteocalcin.[2]

CLASSIFICATION OF RICKETS

Vitamin D-deficiency rickets was once extremely prevalent in Northern Europe and North America, but was nearly eradicated following the introduction of prophylactic vitamin D supplementation in the 1930s and early 1940s. Today the children at risk of developing vitamin D-deficiency rickets are mainly immigrants of Asian origin and children on strict vegetarian diets, cult diets, or other fad diets (Table 1). Provided prompt diagnosis and proper treatment, these children will experience only a short and transient slowing of the growth rate having little impact upon final adult height.

Table 1

Etiologic Classification of Rickets

Vitamin D Deficiency
- Lack of sun exposure
- Dietary vitamin D deficiency
- Vitamin D malabsorption
- Anticonvulsant therapy

Decreased Synthesis of 1,25-$(OH)_2$D
- Vitamin D-dependent rickets type I

End-Organ Resistance
- Vitamin D-dependent rickets type II

Phosphate Deficiency
- X-linked hypophosphatemia
- Tumor-associated hypophosphatemia
- Fanconi's syndrome
- Hypercalciuric hypophosphatemia

Vitamin D-dependent rickets type I is a rare autosomal inborn error of metabolism: 25-(OH)D is not converted to 1,25-$(OH)_2$D and the serum concentration of 1,25-$(OH)_2$D is low. The resulting hypocalcemia leads to secondary hyperparathyroidism, increased phosphate excretion, and subsequent hypophosphatemia. The clinical manifestations, which are similar to those in vitamin D deficiency, usually appear before 1 year of age and include hypotonia and growth failure.[4,5]

1,25-$(OH)_2$D or its synthetic analogue, 1-α-hydroxyvitamin D, is used for treatment. The latter is available in solution and is more convenient for infants and young children. The biologic activity of 1-α-(OH)D is about one half to two thirds that of 1,25-$(OH)_2$D. The recommended doses in treatment of active rickets are 2 to 8 μg/d of 1-α-(OH)D, or 1 to 4 μg/d of 1,25-$(OH)_2$D until radiologically demonstrated healing occurs. This initial treatment takes about 2 to 5 months and is followed by a lifelong supplemental dose of 0.5-2 μg/d of 1,25-$(OH)_2$D. To avoid overtreatment, the serum concentration of calcium and phosphate and the urinary Ca:Cr ratio should be measured periodically. When Ca:Cr exceeds 0.25, the dose should be reduced.

Since replacement therapy in physiologic amounts of 1,25-$(OH)_2$D results in complete correction of the phenotype, including normalization of growth, this disorder or "experiment by nature" probably offers the best human model to assess the effect of 1,25-$(OH)_2$D on growth.

Vitamin D-dependent rickets type II is caused by end-organ resistance to 1,25-$(OH)_2$D due to defective cellular receptor hormone binding and/or expression. At present, 5 different patterns of defects in this rare disorder have been outlined. The clinical hallmarks include early onset of rickets, hypocalcemia, secondary hyperparathyroidism, and very high levels of 1,25-$(OH)_2$D. Alopecia has been noted in about half of the patients. Symptoms, including growth failure, usually appear before 1 year of age.[4]

The patients usually are responsive to high doses of 1,25-$(OH)_2$D, or 1-α-(OH)D, with or without calcium supplementation. Such treatment can heal the rickets, but alopecia never improves. In patients who respond to therapy, the prognosis appears to be good for growth and development. X-linked hypophosphatemic rickets presents with shortness of stature, bowlegs, and hypophosphatemia. Roentgenologic manifestations of rickets are evident by 1 to 2 years of age. Because this is an X-linked dominant trait, males are more severely affected than females.

The biochemical findings are characterized by low serum phosphate, normal or low-normal calcium level, and mild elevation of alkaline phosphatase activity. The serum concentration of PTH is usually normal. The level of 1,25-$(OH)_2$D is also within normal limits, but often this is inappropriately low in view of the hypophosphatemia.

Combined treatment with adequate doses of oral phosphate and 1,25-$(OH)_2$D or 1-α-(OH)D increases growth rates and heals rickets. If treatment starts before 5 years of age, catch-up growth and correction of lower limb deformities can be achieved.[5] The recommended daily phosphate supplement of 1 to 3 g of elemental phosphorus is administered in 4 to 6 divided doses. To avoid gastrointestinal intolerance of phosphate, the initial dose should be small and increased over several months. The initial dose of 1,25-$(OH)_2$D is 15 to 20 ng/kg/d, and is increased over several months to a maintenance dose of 30 to 60 ng/kg/d. During long-term treatment, the serum concentration of calcium and phosphate and the alkaline phosphatase activity should be monitored at regular 1- to 3-month checkups. Radiologic evaluation of the wrists, ankles, and knees, as well as renal ultrasound examinations, should be carried out at 12-month intervals to assess healing of rickets and provide early detection of nephrocalcinosis.

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Obesity in Childhood and Adolescence

Part 2: Pathophysiology, Associations, and Complications

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Childhood obesity now may be the most prevalent nutritional disease in the United States. In the past, the sense of futility that has accompanied unsuccessful attempts at therapy has led many to ignore the disease and its complications. However, recent advances in successful therapy[1,2] suggest that the persistence of the disease is not inevitable. Furthermore, new information regarding its aftereffects provides new insights and poses additional questions regarding its physiology.

PATHOPHYSIOLOGY

Although the similarity in fatness within families[3] and twins[4] is popularly interpreted as evidence that a genetic cause exists for obesity, genetics probably confers only an increased susceptibility to obesity. The causes of obesity are those factors that either increase energy intake, reduce energy expenditure, or impair the regulation of energy balance. In a previous issue of *GROWTH, Genetics, & Hormones* (Vol 7, No. 1), we examined host factors that might increase susceptibility. Basal metabolic rate (BMR) and the thermic effect of food (TEF) appear to be genetically mediated components of energy expenditure, but no significant differences of

Letter to the Editors

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beneficial effects" of an induced growth spurt justifies its use in Turner syndrome. This contention has not been rigorously studied. What I am most concerned about in your article is that you have not played fairly with the studies that are available in the literature and have presented a biased view in what purports to be an even- handed review.

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Associate Professor
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University of Washington
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Seattle, Washington

Response From the Editors

Dr. Sybert believes that the recent review concerning oxandrolone in GGH *ignores a considerable body of evidence that does not support the use of oxandrolone to increase final adult height in Turner syndrome. We respond as follows.*

Several published studies on low-dose oxandrolone therapy in Turner syndrome reported improvement in adult stature. Recent reviews of the use of anabolic steroids to manage Turner syndrome from Joss (1988) and Naeraa et al (1990) imply that oxandrolone may increase ultimate height but acknowledged, as did we in GGH, *that the issue is not settled. Just as importantly, authors in the 7 published articles in the world literature addressing the effect of oxandrolone on final adult height in Turner syndrome reported no adverse effect on final height, and the majority reported a significant improvement, on the average of approximately 4 cm over that expected (ie, Naeraa et al, 1990; Heidemann et al 1987; Joss et al, 1984; Sybert, 1984; Urban et al, 1979; Moore et al, 1977; Stahnke et al, 1985). Stahnke et al did in fact report in abstract form that oxandrolone increased final adult height in Turner syndrome (*Pediatr Res *1985;19:620) although as stated by Dr. Sybert, not in a letter to the editor in the* Journal of Pediatrics *in 1980.*

Regarding the data of Joss and Zuppinger (1984), the conclusions stated in the GGH *review are in accord with the authors' statement, namely, that final adult height was significantly improved in girls treated with oxandrolone for 12 months ($n = 7$; $P<0.05$ for the index of predicted height [IPH] method, but not in comparison to the control group) and 24 months ($n = 8$; $P<0.01$ for IPH and Bayley Pinneau [BP] methods). While true that the final adult height difference at 12 months was not significant between groups (using the BP method), Joss and Zuppinger found that the IPH method is more predictive in girls with Turner syndrome. Thus, our presentation of the data is in full accord with the authors' interpretation of the data.*

We wish to emphasize that all anabolic steroids are not necessarily equal in their actions. Thus, it may be inappropriate to generalize about potential effects of this class of drugs, which is what Dr. Sybert has done in her letter. Dr. Sybert references, for example, the work of Lev-Ran (1977) and Muritano and Job (1985) as evidence against a positive effect of oxandrolone on final adult height. Oxandrolone was not used in these studies. Instead, other anabolic steroids were evaluated. These studies were not included in our review in GGH *since, in our opinion, all anabolic steroids are not necessarily equal in their action.*

Last, and perhaps most importantly, the doses of oxandrolone utilized in patients with Turner syndrome in the Sybert report were 0.13 to 0.29 mg/kg/d and not ≤0.1 mg/kg/d, which is the maximum dose we believe should be used in order to prevent rapid skeletal maturation.

On the basis of these points, we take exception to Dr. Sybert's statements. While the data on the effect of oxandrolone on final adult height in Turner syndrome is inconclusive, the findings with low doses (ie, ≤0.1 mg/kg/d) in girls with bone ages ≥ 8 to 9 years at initiation of therapy suggest final adult height can be increased or, at a minimum, not adversely affected.

The authors sincerely thank Dr. Sybert for expressing her views, and invite others to do similarly.

Robert M. Blizzard, MD
Peter C. Hindmarsh, MD
Richard Stanhope, MD

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either BMR or TEF have been demonstrated among obese and nonobese children or adolescents. The energy spent on physical activity represents the most variable and discretionary component of daily expenditure. Therefore, increases in food intake, reductions in the quantity of energy spent on activity, or impaired regulation of energy balance are the most likely sources of energy imbalance in obesity.

Defective regulation of energy balance appears to cause obesity in patients with hypothyroidism, Prader-Willi syndrome (PWS), or hypothalamic tumors, or after removal of craniopharyngiomas.

Patients who have PWS are short, mildly retarded, sexually underdeveloped, and massively obese. The chromosomes of approximately half of PWS patients carry a partial deletion of the paternally derived chromosome 15 (15q11-13). However, patients with the syndrome who lack the deletion are identical in every respect to those who demonstrate it.[5] This susceptibility to obesity may be increased by reductions in fat-free mass and a low metabolic rate,[6] but obesity is caused by a ravenous appetite. A second small group of patients who demonstrate the same deletion in the maternally derived chromosome 15 develop Angelman's syndrome,[7] a genetic disorder characterized by mental retardation, ataxia, and inappropriate laughter. Obesity in Angelman's syndrome has not been reported. Although both disorders are rare, they offer a rich and fascinating opportunity to understand how genotype affects not only phenotype but behavior.

The majority of obese children lack any evidence that the regulation of food intake is impaired by intracerebral abnormalities. The observation that some obese children gain weight rapidly along curves of unvarying slope suggests that we should not dismiss the possibility that disordered regulation of food intake may account for a small percentage of childhood obesity. Nonetheless, the strong associations of environmental variables with obesity indicate the interaction of strong behavioral determinants.

ASSOCIATIONS

Environmental variables act on the susceptible host to produce obesity. Clues to these variables, although not their action, are found in the environmental variables associated with obesity. Childhood obesity is associated with region, season, and population density. Obesity is more prevalent in the winter and spring, followed by the summer and fall, and is more prevalent in the Northeast, followed in descending order by the Midwest, South, and West.[8] In each region, obesity is more frequent in large metropolitan areas than in any other sampling area. Obesity is also strongly associated with

family variables, such as parental obesity, parental age, family size, socioeconomic class, and parental education.[9] Whether the behaviors linked to these epidemiologic characteristics act to increase food intake, reduce the energy spent on activity, or impair the regulation of energy balance remains unclear.

Television viewing and parental exercise patterns may act to decrease activity. Television viewing is directly related to the prevalence of obesity, and appears to reduce activity and increase food intake.[10] Fatness in children is also inversely related to parental exercise patterns, although fitness is related only to the mother's pattern of exercise.[11]

COMPLICATIONS

Obesity is associated with a variety of physiologic consequences, many of which impact adversely on health. These consequences can be broadly divided into those that result from the auxogenic effects of obesity, the mechanical effects of increased fat mass, and the metabolic effects of increased body fat.

AUXOGENIC EFFECTS

The effects of obesity on growth have been well described. Obese children and adolescents have increased height velocities,[12] although usually ultimate height is not increased. As a result, they are taller than their nonobese peers prior to epiphyseal closure. Bone ages are generally advanced,[13] although probably not in excess of height age. Fat-free mass is also increased,[14] and accounts for the greater BMR observed in obese children and adolescents.[15] However, even when fat-free mass is controlled, metabolic rate appears greater in obese than in nonobese adolescents.[15] The apparently constant rate at which fat-free mass increases with weight gain and decreases with weight loss[16] suggests that adipose tissue changes in concert with fat-free mass. Likewise, the parallel changes in bone age and height velocity suggest that the normal process of growth can be systematically advanced by overnutrition. Nonetheless, the mechanisms by which increases in fatness produce these other effects of growth remain unclear.

Menarche occurs earlier in obese adolescent females. Although earlier menarche has been attributed to the acquisition of body fat necessary to support a pregnancy,[17] this explanation is probably overly simplistic. Evidence to support this hypothesis was derived from estimates of fatness based on weight and height,[18] which are considerably less reliable than direct measures of fatness.[19] Furthermore, changes in relative weight may be less influential than skeletal maturation as a determinant of menarcheal age.[20]

MECHANICAL EFFECTS OF OBESITY

The mechanical effects of obesity can be grouped into effects on bone growth, respiratory status, and psychosocial function. Increased weight bearing generated by obesity causes bowed femurs, Blount's disease (or tibia vara), and slipped caput femoral epiphysis. Increased weight acting on cartilaginous bone in young children produces bowed tibia, and increased bone deposition on the medial side of the proximal tibia. Interestingly, the degree of bowing correlates well with the degree of obesity. Obesity probably acts on the femurs in a similar fashion. Slipped caput femoral epiphysis results from the effects of increased weight across the femoral neck, and the caput of the femur slips on its epiphysis. Although Blount's disease and slipped caput femoral epiphysis may occur in nonobese individuals, the majority of patients affected with these disorders are obese.

The most frequent respiratory disorder associated with obesity is sleep apnea,[21] which is probably caused by increased peripharyngeal fat that narrows the airway. In the pickwickian syndrome, which occurs less frequently, increased intra-abdominal fat decreases the diaphragmatic excursion to produce CO_2 retention. Increased PCO_2 produces CO_2 narcosis and, like sleep apnea, daytime somnolence. In sleep apnea, daytime somnolence results from sleep deprivation incurred by recurrent arousals in response to sleep apnea. In the pickwickian syndrome, daytime somnolence results from CO_2 narcosis. In both disorders, hypoxia may produce cardiac arrhythmia and death.

Peer discrimination and low self-esteem also result from increased body fatness. Our culture is highly sensitized to fatness, and discrimination against the obese begins at an early age.

METABOLIC EFFECTS

In adults, the distribution of fat is directly related to the risk of hypertension, diabetes mellitus, hyperlipidemia, and atherosclerotic cardiovascular disease.[22] Furthermore, the regional deposition of body fat in response to overfeeding appears genetically determined.[23]

In the past several years, a cohesive explanation has evolved to explain the relationship between the regional distribution of fat and its pathologic consequences.[22,24] The distribution of body fat is most commonly assessed by the waist:hip ratio. Excess intra-abdominal fat (android or "apple-shaped" obesity) carries a substantially greater risk of these complications than femoral fatness (gynoid or "pear-shaped" obesity). In android obesity, intra-abdominal fat may be more sensitive to factors that promote lipolysis.[25] High free fatty-acid levels may reduce insulin uptake by the liver. Increased insulin levels may affect blood pressure by increasing sodium resorption by the kidney,[26] and by increasing sympathetic nervous system activity in the heart and peripheral vasculature.[24,27]

Hypertension, abnormal glucose tolerance, and hyperlipidemia also occur in obese children and adolescents, but the frequency of these complications is lower than in adults. Furthermore, the relationship of fat distribution to morbidity in the pediatric age group remains unclear. Central obesity has been associated with the insulin response to a glucose load in normal adolescents,[28] and with blood pressure in normal children[29] and normal and obese adolescents.[30] Because none of these studies controlled for total body fat, it is not clear whether fat distribution affects morbidity more than total body fat.

In males, puberty is accompanied by an increase in skinfolds on the trunk, whereas in females, the increase in body fat is more uniform.[31] However, it is not yet clear whether the pattern of fat deposition increases the likelihood of the metabolic aftereffects of adolescent-onset obesity, or whether the effect of fat localization on morbidity is independent of the degree of excess fat.

The metabolic effects of body fat also play an important role in the genesis of polycystic ovary disease (PCOD).[32,33] PCOD may begin in adolescence, and may affect 4% of women. One third of affected patients have android obesity.[34] Affected women tend to have amenorrhea, hirsutism, and infertility. Fat contains an aromatase that converts androgens, particularly androstenedione, to estrone. In addition, the hyperinsulinemia of obesity may increase androstenedione synthesis by the ovary. The net effect of increased estrone may be to alter the rhythmicity or levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) to produce abnormal stimulation of the ovary and PCOD. Weight loss may reverse the cycle entirely.[32,33] In some families, PCOD appears to be inherited as an autosomal dominant trait. However, primary abnormalities of insulin action that lead to obesity are suggested by the finding of acanthosis nigricans, insulin resistance, and polycystic ovaries in a woman with an abnormality of the insulin receptor.[35] The finding that 5% of all

women with PCOD had acanthosis nigricans, obesity, and hyperinsulinemia[36] suggests that this defect may be among the most common genetic syndromes associated with obesity.

SUMMARY

The diversity of central and peripheral host factors that can influence the susceptibility and causes of obesity is aptly illustrated by PWS and PCOD. The extent to which these factors operate in the general population and the behavioral linkages that connect the epidemiologic variables associated with obesity to these or other host factors remain uncertain. However, these observations suggest that even the prevalent and apparently mundane disease of obesity is still surrounded by a multiplicity of questions, the answers to which promise new insights into the metabolic interactions of genetics and growth.

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Annual Meeting of the Endocrine Society: Highlights

Dr. Seymour Reichlin opened the symposium with an elegant review of the endocrine physiologic factors that interact in response to stress. The entire brain-hypothalamic-pituitary end-organ axis is activated. Much of the presentation centered on the neuroendocrine aspects, with emphasis on the interactions between the neuroendocrine axis for corticotropin release and the immune regulatory system. Interleukins 1 and 6 (IL-1 and IL-6) are particularly involved in the activation of the neuroendocrine system in response to stress; however, it is not a global activation. Data were presented to show that IL-1B activation through specific IL-1 receptors differed substantially from the bacterial lipopolysaccharide-stimulated IL-6 release of rat anterior pituitary cells.

Exciting new insights into the interactions among the great integrative systems of physiology (endocrine, neuroendocrine, and immunologic) will be forthcoming in the near future as some of the endocrine, paracrine, and possibly autocrine activities are uncovered and the corresponding physiologic mechanisms described.

Dr. Larry Parker reviewed the past decade's data on the elusive pituitary factor that stimulates adrenal androgen production. This factor, alternatively called AASH (adrenal androgen-stimulating hormone) and CASH (cortical androgen-stimulating hormone), reportedly is synthesized in the anterior pituitary gland, differs from corticotropin, and is regulated by mechanisms that differ from those described for corticotropin. Partial amino-acid sequence data were presented that indicate/identify with a part of the joining peptide of pro-opiomelanocortin (POMC), but at present there are no unequivocal chemical or biologic data to consider the AASH (activity) a single, distinct entity with a

proven structure and physiologically relevant activities.

Dr. Lynn Loriaux delivered another of his comprehensive, well-balanced lectures on the vast experience of the National Institutes of Health (NIH) group using corticotropin-releasing hormone (CRH). The subjects chosen — patients with Cushing's syndrome and those with depression — reflect the great numbers of patients referred to the NIH and the close working relationship between the psychiatrists and the endocrinologists. Responses to exogenous CRH are abnormal in both groups of patients. Exogenous CRH is probably more helpful in categorizing patients with Cushing's syndrome into specific pathophysiologic entities, eg, ectopic Cushing's syndrome and Cushing's disease. Emphasis was placed upon the clinical presentation and the sum of the biochemical and imaging tests rather than upon the CRH (or any other single) test.

Suffice it to say that there remains significant biologic variability even within seemingly single biologic entities and that the CRH test is but another "window" into the disordered neuroendocrine axis for adrenal function in both of these syndromes.

Dr. Louis Underwood opened the symposium with a review of the expanding role for growth hormone (GH) in non-GH-deficient states. Great controversy still exists in the definition of some of these states and upon the efficacy of both short- and long-term hormonal treatment. GH is efficacious in accelerating growth in girls with Turner syndrome and may increase adult height in these girls. A particularly exciting new use is to increase the efficient harvesting of ova for in vitro fertilization.

Dr. Richard Fine presented an update of his and other studies using GH in growth-retarded children with chronic renal insufficiency. There are a number of relatively short-term studies that indicate a growth-promoting effect of this hormone in these children. None has followed children to adult height to see if the predicted gains actually continue to final height. The data were exciting, and the role of GH treatment in this and other conditions is but one of the avenues being explored.

Alan D. Rogol, MD, PhD

Abstracts From the Literature

Pubertal Growth in Chronic Renal Failure

This paper analyzes the height growth of 15 boys and 14 girls with end-stage renal failure first studied before puberty and followed at 3- to 6-month intervals until growth ceased or nearly ceased. The height data were smoothed by the kernel estimation method, which is a form of moving average. The records were from Heidelberg, and the curves were compared with those from the Zurich Longitudinal Growth Study. This made possible a comparison with late normal maturers as well as with the average maturers in a normal growth study.

The start of the pubertal growth spurt was delayed by 2.5 years in both the girls and boys, and its duration and intensity were also very significantly reduced, with the mean height gain at around 50% of that observed in the late-maturing control group. However, mean height at the onset of the spurt was approximately the same as that in the late-maturing control group. The data indicate that most patients with end-stage renal failure occurring before or during puberty irreversibly lose growth potential. Renal transplantation did not consistently improve pubertal growth.

Schaefer F, Seidel C, Binding A, et al. *Pediatr Res* 1990;28:5.

Editor's comment: *This paper is particularly striking because of the use of the kernel estimation method, which, in my opinion, is currently the most advanced technique for analyzing growth curves. Since it is nonparametric, it is particularly applicable in cases of growth disorder, and this paper constitutes a real model for other research workers studying growth in chronic disease. It is interesting that in the patients with renal failure, puberty did not start until their height had reached virtually that of the controls when they started puberty; however, by this time height velocity was far below normal and the subsequent pubertal spurt was very much reduced. Such a fine analysis does require many measurements of height to be made during the growth period but results in a much better understanding of the dynamics associated with the disorder than has previously been possible.*

James M. Tanner, MD

In Future Issues

Insulin-Like Growth Factors and In Utero Growth
by Joseph D'Ercole, MD

Testis-Determining Factor: An Update
by Barbara C. McGillivray, MD

GH Deficient-Like Syndromes and Their Etiologies
by William H. Daughaday, MD

Intrauterine Growth Restriction Revisited
by Joseph D. Warshaw, MD

X Inactivation Is Not Really Complete Inactivation of the Whole Chromosome

X-chromosome inactivation in mammals is a regulatory phenomenon in which gene expression from 1 of the 2 X chromosomes in female cells is inactivated, resulting in dosage compensation for X-linked genes between females (with 2 X chromosomes) and males (with only 1). However, a series of papers recently indicates that the inactivated X is not completely silent. There are now at least 4 regions on the short arm and 2 regions on the long arm of the inactivated X that contain actively expressed genes.[1] One of these genes, cloned by Fisher and colleagues, codes for a ribosomal protein, which has a homologue on the Y chromosome. The authors suggest that some or all of the features of Turner syndrome (45,X) could be the result of haploinsufficiency for this type of protein.[2] Another interesting area on the long arm of the X chromosome is a region that has been identified by Brown and colleagues as the putative X-inactivation center (XIC), mapped to Xq13.[3] This is believed to be a locus that is blocked by a *trans*-acting factor in the active X, ie, unless it is blocked the chromosome is inactivated.[4] Brown et al have also identified a gene that produces an RNA transcript only from the *inactive* copy of the X chromosome. In addition, this gene, called XIST (for X inactive-specific transcript), appears to lie in the same region as the putative XIC. Thus, it is a candidate for a gene either involved in or uniquely influenced by the process of X inactivation.[5]

Editor's comment: *The mechanism by which the genes on the inactive copy of the X chromosome are silenced has long been an enigma. The mystery has been heightened by the discovery that many genes escape inactivation, ie, it is not an "all-or-none" phenomenon. The localization of the XIC and of a gene that is expressed only by the inactive copy of the X chromosome will hopefully provide tools with which to dissect the process of X inactivation on a molecular level.*

Judith G. Hall, MD

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IGF-2 and IGF-2 Receptor: Evidence for Genomic Imprinting and Complementarity in Parental Genetic Contribution to Growth

A compelling body of evidence from widely diverse areas of research suggests that the expression of some genes depends upon their parental origin, ie, whether they have been inherited from the mother or from the father. Recent developments in studies of a growth factor gene and its receptor indicate that genomic imprinting is involved in the regulation of expression of these genes.

Horton reported in the March 1991 issue of *GGH*[1] on the experiments by DeChiara et al that provided direct evidence for the role of insulin-like growth factor 2 (IGF-2) in antenatal growth.[2] These experiments demonstrated that transgenic mice carrying 1 normal and 1 disrupted copy of the IGF-2 were much smaller than normal controls with 2 functional copies of the gene. Subsequently, these same authors found that when the disrupted IGF-2 gene is transmitted to offspring through the male germ line, progeny are growth deficient, as shown previously. However, when the disrupted gene is transmitted maternally, the heterozygous offspring (ie, 1 normal copy and 1 disrupted copy) are phenotypically normal. They also found that only the paternal allele is expressed in embryos, while the maternal allele is silent. In addition, homozygous offspring carrying 2 copies of the disrupted gene were indistinguishable from the heterozygotes. They thus concluded that the IGF-2 gene is subject to parental imprinting.[3]

At approximately the same time, Barlow et al began studying the T-associated maternal effect (*Tme*) mutation in mice. This defect is nuclear-encoded, and embryos that inherit a deletion of the *Tme* locus from their mother die at day 15 of gestation. Barlow and colleagues found that the genes for the IGF-2 receptor (IGF-2r) lie within the region of this deletion, thus making it a candidate for the *Tme* gene. They also demonstrated that embryos express IGF-2r only from the maternal chromosome, and it is therefore paternally imprinted.[4]

Editor's comment: *These studies of a growth factor gene and its receptor provide further support for the theory of genomic imprinting, and indicate that the maternal and paternal genetic contributions may play complementary roles in growth regulation, acting to balance each other so that growth proceeds in a controlled fashion but does not proceed beyond certain carefully regulated boundaries. The identification of specific genes that are imprinted also provides excellent tools for determining the molecular mechanism(s) of imprinting.*

Judith G. Hall, MD

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The Protective Effect of Growth Hormone on Steroid Damage to Bone and Cartilage in Mice

Glucocorticoid therapy in children and young animals causes growth deceleration and degenerative changes of bone and cartilage. To evaluate the possible therapeutic effect of growth hormone (GH) in the presence of excessive glucocorticoids, 3-week-old female ICR mice were treated IM for 4 weeks as follows (n = 5 each group): (1) Control, saline. (2) Dexamethasone (DEX),1 mg/kg/d. (3) recombinant GH (rGH) 1 mg/kg/d. (4) bovine GH (bGH), 1mg/kg/d. (5) DEX + rGH. (6) DEX + bGH. Tibiae and vertebrae were analyzed for morphometric and biochemical parameters. Growth, as measured by weight and tibial length, was compromised in the DEX group, but reversed to control growth rate by rGH and bGH. Epiphyseal growth plate width was 295 ± 17 μ in the DEX group, 360 ± 26μ in the DEX ± rGH (P<0.01), and 352 ± 34 μ in the DEX + bGH group (P<0.02). Cortical bone width was 265 ± 22 μ, 292± 27 μ and 285 ± 31 μ, respectively (P>0.05). Trabecular bone volume, as percent of total bone volume, was 9.8 ± 2% in the DEX group, 22.1 ± 4.7% in the DEX + rGH group (P<0.01), and 18 ± 2% in the DEX + bGH group (P<0.001). Minerals from the lumbar vertebrae after ashing were 27 ± 2.3 mg, 34 ± 2.3 mg (P<0.01), and 35 ± 3 mg (P<0.01), respectively. Bone soluble protein was 14.9 ± 2.4 μg/mg, 20.7 ± 0.56 μg/mg (P<0.01), and 20.6 ± 0.6 μg/mg of bone (P<0.01), respectively. Bone acid phosphatase was 0.5 ± 0.012 U/g bone weight in the DEX group, 0.9 ± 0.027 U/g in the DEX + rGH group (P<0.001), and 0.74 ± 0.054 U/g bone in the DEX + bGH group (P<0.001). Bone alkaline phosphatase was 1.56 ± 0.09 U/g, 4.05 ± 0.05 U/g (P<0.001), and 4.04 ± 0.02 U/g (P<0.001), respectively. It is concluded that GH treatment to a large extent prevents the damage inflicted on bone and cartilage by dexamethasone.

In summary, the increases in weight and bone length reflect the growth-saving effect of GH. The increases in trabecular bone volume and epiphyseal growth plate width indicate an increase in bone formation, and the protein and phosphatase increases reflect the protective effect of GH on the tissue destruction by dexamethasone.

Altman A, Silbermann M, Hochberg Z. 30th Annual Meeting of the ESPE, 1991.

Editor's comment: *This abstract was published in the proceedings of the 30th Annual Meeting of the European Pediatric Endocrine Society (EPES). It was the basis for a special presentation and an award from the EPES. Dr. Raphael Rappaport presented the paper. The importance of the paper is that it is another of a few (Horber et al,* J Clin Invest *1990;86:265; Horber et al,* Diabetes *1991;40:141) that suggest GH can overcome the antianabolic and growth-inhibiting effects of glucocorticoids. What a blessing it will be if GH can reverse the devastating effects of chronic corticosteroid therapy in childhood. Before capitalizing upon the substance of these reports, however, investigators are urged to set up controlled protocols. Now is the time to do exactly that. It is not the time to muddy the therapeutic waters.*

Robert M. Blizzard, MD

The Strange Case of Fragile X Syndrome: Increased Mutation Frequency, Increased Fragment Size, and/or Genomic Imprinting?

The fragile X syndrome may be the most frequent cause of inherited mental retardation; the incidence is about 1 in 1,500 males and 1 in 2,500 females.[1] It is a most puzzling syndrome for a number of reasons. Affected males have been characterized as having a relatively normal phenotype except for megalotestes and the presence of a large head, high prominent forehead, prominent jaw, and large protruding ears. Affected carriers have been diagnosed only by the finding of a characteristic abnormality in which the tip of the long arm of the X chromosome seems to be connected to the rest of the chromosome by a slender thread when the cells are cultured under specific conditions. Such chromosomes are easily broken — hence the name fragile X. This gross chromosomal change is rarely evident, however, in asymptomatic carriers of the defect.[2]

The inheritance pattern of the fragile X syndrome seems bizarre in terms of traditional expectations for X-linked diseases. Surprisingly, between 20% and 50% of males who carry the fragile X mutation are asymptomatic. These asymptomatic male carriers can pass the gene along to their daughters, who are also asymptomatic, as would be expected for an X-linked disease. But in the third generation, the children of those daughters — both males and females — are likely to express the facial features of the syndrome,[2] and the affected males will have megalotestes.

Recently, 2 groups have independently identified the site of the fragile X mutation.[1,3] When Yu and colleagues used their probe, specific for the fragile X region, on the chromosomal DNA of normal and fragile X genotypic individuals, alterations in the mobility of the sequences detected by Southern blotting were found only in fragile X genotype DNA. These sequences were of an increased size in all fragile X individuals and varied within families, indicating that the region was unstable.[3] Oberle and colleagues have linked the phenotypic expression of the syndrome to abnormal cytosine methylation of a single CpG island, at or very near the fragile site. Probes adjacent to this island detected very localized DNA rearrangements that constituted the fragile X mutations, within a 550-base pair, GC-rich fragment. They found that normal male carriers had a 150- to 400-base pair insertion that was inherited by their daughters either unchanged or with small differences in size. Fragile X-positive individuals in the next generation had much larger fragments that differed among siblings and showed a generally heterogeneous pattern, indicating somatic mutation. The mutated allele appeared unmethylated in normal male carriers, methylated only on the inactive X chromosome in their daughters, and totally methylated in most fragile X males. They thus concluded that expression of the fragile X syndrome appears to result from a 2-step mutation as well as a highly localized methylation.[1] Kremer et al have further characterized this region, and have found that the instability was localized to a trinucleotide repeat, p(CCG)n. The sequences flanking this repeat were identical in normal and affected individuals. The break points in 2 somatic cell hybrids constructed to break at the fragile site also mapped to this repeat sequence. The repeat exhibits instability both when cloned in a nonhomologous host and after amplification by the polymerase chain reaction.[4] These results suggest variation in the trinucleotide repeat copy number as the molecular basis for the instability and possibly the fragile site. This would account for the observed properties of this region in vivo and in vitro. These studies do not explain why such an unstable sequence would be maintained in the genome, let alone further amplified in fragile X pedigrees. Nor

do they address the issue of methylation of the region in fragile X syndrome individuals. The composition of the unstable sequence, which contains many targets for methylation, provides a link between the instability seen in the fragile X genotype and the methylation of this region associated with the fragile X syndrome phenotype.[4]

Editor's comment: *The discovery of the fragile X site will allow testing for both affected and asymptomatic carriers of the mutation. This will aid in prenatal diagnosis and in genetic counseling for carriers who are contemplating having children. Because it often had been impossible in the past to detect asymptomatic carriers of the fragile X mutation, the ascertainment of complete pedigrees has been difficult. It represents a new type of mutation in which amplification of an abnormal site occurs. Time will tell how many other disorders will share this mechanism of disease production.*

Judith G. Hall, MD

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Insulin-Like Growth Factors and Their Binding Proteins in Human Fetal Serum: Relationships With Fetal Growth

Cord blood, obtained by direct transparietal puncture of the umbilical cord for purposes of prenatal diagnosis, allowed this study of 119 human fetuses between 20 and 37 weeks of gestation, among which 103 were of normal size for gestational age and 15 had intrauterine growth retardation. Neonatal cord blood of 37 normal-term newborns also was studied. The authors measured in these sera: insulin-like growth factor 1 (IGF-1) by radioimmunoassay, IGF-2 by competitive protein binding assay, and their specific carrier proteins (IGF-binding proteins [IGFBPs]) by binding to ^{125}I-labeled IGF-1 after separation by elution from a column of Ultragel. The respective association of IGF-1 and IGF-2 with their BPs was determined by electrophoretic separation obtained with western-ligand blotting. Placental lactogen (PL) was measured by radioimmunoassay.

The serum levels of both IGFs were steady from 27 to approximately 33 weeks of gestation, close to 50 ng/mL for IGF-1 and 350 ng/mL for IGF-2. Thereafter both increased to reach, at term, values 2 to 3 times higher. The profiles of these age-related changes were roughly parallel along an exponential regression curve. PL in the serum of fetuses followed a similar curve. Significant correlations were found between the levels of PL and those of both IGFs, suggesting that PL may be involved in the regulation of circulating IGF-1 and IGF-2 in the human fetus.

Total concentrations of the IGFBPs in the fetuses were low. Binding activity was approximately half of that found in normal adults. Qualitatively, the BP profiles in fetuses resembled those of patients with growth hormone deficiency, with small amounts of 41.5- and 38.5-kd forms, contrasting with relatively increased 34-kd and 30-kd forms. The distribution of the IGFBP in the newborns' complexes showed a low proportion of the 150-kd complex, which is the predominant form in normal children and adults.

The relationships with fetal weight were studied using both echographic data and the birth weight. The levels of IGF-2 during the latter months of gestation did not relate to the fetal weight. However, IGF-1 levels in the fetuses with subnormal weight were significantly lower than in those of the same age whose weight was appropriate, mainly after 25 weeks. This suggested to the authors that during the 3 or 4 last months in utero, IGF-1 but not IGF-2 is involved in the control of fetal size.

Lassare C, et al. *Pediatr Res* 1991;29: 219-225.

Editor's comment: *This work yields new data of significance for understanding how human fetuses grow during the second half of gestation. Several points are to be stressed: IGF-2 levels in fetal serum are 4 to 7 times higher than the levels of IGF-1. Both rise in parallel fashion in the late intrauterine period, contrary to what occurs in the rat fetus, whose hepatic synthesis switches from IGF-2 to IGF-1 at the end of gestation. PL is likely to be a regulatory factor; the amount of specific IGF carrier proteins, their profile, and the complexes that they form with IGFs suggest a physiologic situation of high bioavailability of IGF at a time when it is probably most needed. In addition to these physiologically relevant results, the data obtained by comparing small-for-gestational age and normal-for-gestational age fetuses confirm some previous studies strongly suggesting that insufficiency of circulating IGF-1 is found in fetal hypotrophy. However, this does not preclude the role of local factors having paracrine or autocrine effects. Fetal growth, its regulatory mechanisms, and especially the role of the placenta, the various factors that may cause intrauterine growth retardation, and the changes occurring during the last 3 months of pregnancy are considered in this report. This work demonstrates that clinical investigations in this field may provide very relevant contributions.*

Jean-Claude Job, MD

Editor's comment No. 2: *This article emphasizes that IGF-1 may play a more important role in fetal growth than IGF-2. The studies of DeChiari et al which were abstracted in* GGH *(1991;7(1):13) suggest IGF-2 is more important than IGF-1, at least in mice. What is the relationship between fetal growth, IGF-1, and IGF-2, and other growth factors in humans? In rodents? Dr. D'Ercole will address these issues in the March issue of* GGH *(8:1).*

Robert M. Blizzard, MD

Function of GH–IGF-1 Axis in the Profoundly Growth Retarded Diabetic Child: Evidence for Defective Target Organ Responsiveness in the Mauriac Syndrome

Mauriac syndrome consists of a triad of poorly controlled insulin-dependent diabetes mellitus (IDDM), profound growth retardation, and hepatomegaly, and is seen relatively rarely in 1991. However, it still occurs and the etiology of the growth failure defies explanation. Mauras et al attempted to determine whether the growth retardation was secondary to decreased or abnormal growth hormone (GH) secretion and/or action. The study described compared data in 2 patients with Mauriac syndrome with data from 5 age-matched diabetic boys who were growing well.

Overnight GH profiles in the Mauriac patients and in the normally growing diabetics were similar in respect to mean 12-hour GH concentration, pulse amplitude, and pulse frequency and did not change during an acute normalization of the serum glucose overnight. The GH-binding proteins (relative binding) were similar in all patients and comparable to those in normal nondiabetics. Insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein (IGFBP) concentrations were comparable in both groups of patients. One patient with Mauriac syndrome treated with GH failed to respond with increased growth velocity (GV) over a 1-year period.

The authors concluded that the data suggest a GH-resistant state either secondary to impaired bioactivity of IGF-1 or a defect at or distal to the IGF-1 receptor.

Mauras N, et al. *Metabolism* 1991;40:1-6.

Editor's comment: *These studies are very important even though no explicit explanation is found for the severe growth failure manifested in patients with the Mauriac syndrome. I have been perplexed through the years when observing these patients. I still am, but thanks to Mauras and colleagues we now know that the complex cascade of events that controls growth, starting with a positive hypothalamic signal, followed by pulsatile GH release, and GH binding to its protein through IGF-1 and IGFBP generation, is intact in these youngsters. If the defect is truly at or distal to the IGF-1 receptor, advances in technology should help us elucidate that defect in years to come.*

Robert M. Blizzard, MD

Repeated Subcutaneous Administration of Recombinant Human Insulin-Like Growth Factor 1 (IGF-1) to Human Subjects for 7 Days

This paper presents the results of a preliminary trial of insulin-like growth factor 1 (IGF-1) obtained by recombinant DNA technology in adult volunteers. Although biosynthesis of human IGF-1 was reported in 1986 and several experimental studies have been done, human trials have been delayed because of the risk of insulin-like metabolic effects.

This study was conducted in 9 healthy young adult volunteers and 2 growth hormone-deficient (GHD) young adults who had not received GH for 3 years. IGF-1 (0.1 mg/kg) was injected sc after breakfast into 2 patients and into 6 of the healthy subjects. The other 3 controls received placebo. Dietary intakes were of an average type and were controlled. The IGF-1 of 97% purity was dissolved in saline just before use. Blood glucose, plasma insulin, and plasma IGF-1 were measured before treatment and then after the first and seventh injections of recombinant IGF-1 (rIGF-1). The method used for assaying total IGF in plasma was radioimmunoassay after acid ethanol extraction. Free IGF-1 was measured after separation on a Sep Pak C 18 cartridge.

Free IGF-1 increased after injection but rapidly decreased thereafter. Total IGF-1 increased later to reach a peak 2 hours after injection and remained elevated above the baseline for 6 to 24 hours, probably due to its binding on carrier proteins. The GHD patients had lower total IGF-1 levels than the normal subjects following injection of IGF-1, possibly since they lacked the GHD main binding protein (IGFBP-3) for IGF-1. The effects of the seventh injection were similar to those of the first, although the basal levels of free and total IGF-1 were slightly higher at the end of the 1-week trial than at the onset of the trial.

Significantly, rIGF-1 (0.1 mg/kg) injected sc after breakfast did not induce hypoglycemia in the GHD patients or healthy subjects. There was only a small decrease in the fasting glucose level. The authors stress this point since in a previous trial using a slightly higher dose of rIGF-1 (0.12 mg/kg) given after an overnight fast they had observed a drop in blood glucose to <50 mg/dL in 3 of 5 normal subjects.

Serum insulin levels decreased slightly at the end of the trial. The authors discussed the role of 2 possible factors: a direct effect of IGF on insulin release by the pancreatic islets and an indirect effect mediated by the slightly lower level of fasting blood glucose. They concluded that the repeated administration of rIGF-1 (0.1 mg/kg) after breakfast appeared to be safe and to have some biologic effect.

Takano K, et al. *Growth Regulation* 1991; 1:23-28.

Editor's comment: *Recombinant IGF-1 is now available for clinical investigation. Among the questions to be asked are (1) will IGF-1 therapy increase growth in syndromes of GH insensitivity (eg, Laron's dwarfism); (2) will IGF-1 replace GH as the favored therapeutic agent in the treatment of GHD or other types of growth failure; and (3) is IGF-1 safe, or will the insulin-like effects preclude its use?*

After 7 days of daily injections at the dose used (0.1 mg/kg), the blood glucose values were not depressed significantly. However, as reported by Walker et al (N Engl J Med *1991; 324:1428), a continuous intravenous injection of 0.05 mg/kg daily did produce mild chemical but clinically asymptomatic hypoglycemia in 1 patient with Laron's dwarfism (a GH-resistant syndrome). The patient retained nitrogen, developed hypercalciuria, and had decreased phosphate and sodium excretion.*

The tentative answers to the questions are (1) we don't know as yet, but preliminary data indicate that GH will probably increase growth in the GH-resistant syndrome of Laron's dwarfism; (2) currently, there seems to be no theoretical advantage to using IGF-1 to treat GHD; and (3) clinical hypoglycemia secondary to the use of IGF-1 at doses that are metabolically active is probably not going to be a problem.

Jean-Claude Job, MD

Is *SRY* the Testis-Determining Factor?

The initiation of male development in mammals requires 1 or more genes on the Y chromosome. A recently isolated gene, termed *SRY* in humans and *Sry* in the mouse, has many of the genetic and biologic properties expected of a Y-located testis-determining factor (TDF) gene. The *SRY* gene lies in the 35-kb interval near the Y pseudoautosomal boundary. A number of genes have been isolated in this region and it is the *SRY* gene that is consistently present in XY individuals who are male.[1] Abnormalities in the *SRY* gene have been found in several XY females.[1-3] In addition, the *Sry* gene is expressed during testes development in the mouse.[4] Expression of the *SRY* gene is confined to gonadal tissue. The gene is highly conserved across species.

Recently, Koopman and colleagues[5] have demonstrated that the Sry gene contained in a 14-kb genomic DNA fragment is sufficient to induce testis differentiation and subsequent male development when introduced into chromosomally female mouse embryos. Sequencing failed to detect any other gene sequences in the 14-kb fragment. Since this fragment alone was able to cause sex reversal, the authors postulate that it contains the entire *Sry* gene, including all of the regulatory elements required for appropriate embryonic expression. Interestingly, these phenotypically male XX mice proved to be sterile despite normal mating behavior, as have all other XX males tested.

Editor's comment: *The search for TDF has been a long and hard one. While there may be multiple factors involved in complete sexual differentiation (as evidenced by the failure of maturation of the germ cells in the XX males),[5] these recent developments provide compelling evidence that the* Sry *gene is necessary and sufficient for external male development, at least in the mouse. This topic will be covered more fully by Dr. B. McGillivray in* GGH *Volume 8, Number 2.*

Judith G. Hall, MD

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The Gene of Insulin-Like Growth Factor 1 and the Gene Cluster of Human Growth Hormone in Children With Constitutional Short Stature

Abnormality of the growth hormone (GH) gene cluster or of the gene of insulin-like growth factor 1 (IGF-1) has long been considered as a thematically possible cause for constitutional or familial short stature. A study of 17 children from 10 constitutionally small families fails to support this hypothesis The children studied (10 males and 7 females) were ages 4.1 to 11.9 years, with a height -2.5 to -3.6 standard deviations (SD) and a mean height velocity -0.35 ± 0.25 SD. They had no detectable cause of short stature other than having 1 parent with a height insufficiency of -2.5 to -3.4 SD. They were compared with 3 groups of controls: 25 adults of normal stature, 50 unrelated children of normal height, and 60 unrelated children with isolated growth hormone deficiency (IGHD).

The technique used for genetic study was Southern blotting and linkage analysis of restriction fragment length polymorphism (RFLP) on peripheral blood leukocytes.

The patterns of hybridizing DNA fragment generated by 7 different restriction enzymes did not show any difference between the group of constitutionally short children and the 3 control groups. Linkage analysis excluded the possibility that the IGF-1 gene would exert a dominant effect in these short children.

The frequency of the different GH gene cluster haplotypes within the 10 families having 1 parent and 1 child constitutionally short did not show a significant difference between the short and the non-short members, nor between constitutionally short individuals and the various controls. These data, and an analysis of previously published studies in the same field, led the authors to conclude that structural abnormalities of the GH gene cluster detectable by restriction endonuclease analysis are only very rarely a cause for growth failure.

Mullis PE, et al. *Pediatr Res* 1991; 29:412-415.

Editor's comment: *Hereditary short stature has been the subject of several recent studies, all more or less related to the question of the possible effects of additional GH in these endocrinologically normal children demonstrating sometimes extreme height insufficiency.*

Most studies based on hormonal measurements, evaluation of cell receptivity, and other factors participating in the "growth regulation cascade" have not yet given any answers to the questions raised about constitutionally small people. Even if some authors have found a somewhat lower level of GH secretion or circulating IGF-1 in the "short" groups, up to now it has not been considered as a main characteristic of this human group. Since it obviously relates to some kind of hereditary transmission, genetic shortness must have its cause in the genome or its expression. The negative results of the research done by Mullis and colleagues are important, since they strongly suggest that this genetic cause is not a deletion affecting the GH gene cluster or the structural gene of IGF-1. There are still many "candidates" inside the human genome. Even if there is a gap between the clinical facts and the known animal models of body size heredity today, constitutional or familial short stature remains an important field for clinical research.

Jean-Claude Job, MD

Editor's comment No. 2: *The term constitutional short stature should be discarded. It is confused with constitutional delay of growth (and puberty later). Genetic or familial short stature (FSS) are acceptable terms and FSS is the entity discussed in this manuscript.*

Robert M. Blizzard, MD

Evidence of Hypothalamic-Pituitary Thyroid Abnormalities in Children With End-Stage Renal Disease (With Growth Retardation)

The authors report studies of thyroid hormone levels and thyrotropin (TSH) response to thyrotropin-releasing hormone (TRH) injection in 9 children (7.5 to 17 years of age) with end-stage renal disease (ESRD). All were receiving either hemodialysis or peritoneal dialysis. The bone age (BA) was at least 2 1/2 years less than the chronologic age. Height, expressed as standard deviations (SD) from the mean for age and gender, was 2.9 ± 1.1 SD below the mean. The mean growth velocity was 2.8 ± 1.6 SD below the mean for BA and gender.

As listed in the table below, thyroxine (T_4) levels were low in almost half of the patients and free thyroxine levels were low in all. However, triiodothyronine (T_3) levels were in the normal range. All but 1 patient had basal TSH levels within the normal range. Three patients had a deficient TSH response to TRH. The TSH response was prolonged in all 9. The mean (± SD) nocturnal TSH surge was 50 ± 68%, as compared with a mean of 124% in the normal controls. The TSH surge was below the normal range in 5 of 8. Serum free T_4 values correlated with the nocturnal TSH surge. The authors conclude that their findings support the hypothesis that some patients with ESRD have central hypothyroidism.

Pasqualini T, et al. *J Pediatr* 1991; 118: 873-878.

Editor's comment: *These authors have extended other studies concerning circulating thyroid hormones in children with ESRD. The study nicely demonstrates that several of the parameters we use to measure low thyroid function indicate central hypothyroidism exists in some patients with ESRD. In addition, the authors commented that the alterations in thyroid hormone levels observed in these patients differ from those found in patients with altered thyroid function due to other forms of acute or chronic illness. In the latter, there frequently is a decrease in total serum T_3 concentration and a rise in serum reverse T_3 concentration. In ESRD there appears to be a predominant decrease in T_4 as opposed to T_3 levels. A plausible explanation for the low level of circulating thyroid hormone seems to be that patients with ESRD have a defect in the pituitary secretion of TSH. Because the patients predominantly had prolonged elevations of TSH following TRH administration, the primary site of the metabolic defect is presumed to be the hypothalamus.*

My interpretation of these data is that thyroid function is probably abnormal (low in many instances) secondary to a central deficiency of TSH release or production. The growth delay these patients experience is not necessarily related to this, although it might be. In such patients, a trial of thyroid hormone treatment and observation of its effect, if any, on growth would be worthwhile.

Robert M. Blizzard, MD

	T_4 (nmol/L)		FT_4 (pmol/L)		T_3 (nmol/L)	
	Average	No. Below Normal Range	Average	No. Below Normal Range	Average	No. Below Normal Range
Normal	118.7 ± 22	0	18 ± 49	0	2.3 ± 0.5	0
ESRD (9)	76.7 ± 16.9	4	10.4 ± 2.6	9	1.6 ± 1.3	0

The Somatostatin Analogue Octreotide: Possible Use for the Treatment of Excessive Body Growth: A Compilation of 3 Reports

An early report of preliminary results was obtained with the long-acting somatostatin (SRIH) analogue, octreotide, in 7 adolescents with excessive height and height velocity[1] and was summarized in *GGH* (Vol 6, No. 2). Another report was published a few weeks later.[2] It included 6 constitutionally tall girls, ages 12.7 to 13.6 years, with a mean predicted height of 184.5 ± 4.8 cm, and 4 constitutionally tall boys, ages 14 to 15.5 years, with a mean predicted height of 198.7 ± 6.2 cm. All were treated with twice daily subcutaneous (sc) injections of 150 μg of octreotide for 6 to 12 months. The 24-hour mean integrated concentration of growth hormone (GH) decreased from 5.3 to 3.6 ng/mL/min after 6 months and to 3.9 ng/mL/min after 12 months, with large individual variations. The GH peak following injection of the peptide thyrotropin-releasing hormone (TRH) reached 8.9 ng/mL before treatment and dropped to 3.1 and 2.7 ng/mL after 6 and 12 months, respectively, with octreotide. The GH response to GH-releasing hormone (GHRH) did not change significantly. The clinical effects included a decrease in the mean growth velocity from 7.1 cm/yr to 2.7 cm/yr after 6 months and to 2.4 cm/yr after 1 year. The mean bone maturation progressed 1 year after 6 months of treatment and more than 2 years after 12 months. A mean reduction of

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predicted adult height (according to Bayley-Pinneau) of 4.9 ± 2.9 cm resulted. These results are in agreement with those of the previously related trial.[1] Although the tolerance of octreotide was said to be good, diarrhea during the first 10 days of treatment occurred in the 10 adolescents, and persisted in 1. Three reported nausea for 5 minutes following the injection. Transitory biliary microlithiasis was found in 1 female patient.

A recent comprehensive review[3] gives data on the pharmacology of octreotide. This somatostatin analogue has a half-life of 113 minutes after sc injection. In adult acromegalic patients, it is able to suppress GH secretion for 6 hours. No significant rebound secretion of GH occurs, and SRIH has few inhibiting effects on insulin secretion. The known effects of the analogue in acromegaly and in various cancers are summarized, and the results obtained in constitutionally tall adolescents in the first published study are reported.[1] It is believed that octreotide not only inhibits or suppresses the pituitary secretion of GH for several hours but also may act directly on bone formation. The presence of high-affinity binding sites for ^{125}I-labeled octreotide in long bones of neonatal rats and the inhibiting effect of octreotide on the forskolin-induced adenylate cyclase activity in bone cell preparations from newborn rats have been demonstrated. The number of receptors decreases with age in the rat. This leads one to question if the clinical results obtained with octreotide in excessively tall adolescents could result in part from a direct effect of the peptide on long bone formation.

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Editor's comment: *To treat or not to treat excessive growth at adolescence is a controversial question. The ethics for such treatment, the means proposed, the efficacy, and the safety have long been discussed — without developing a consensus. The 2 clinical trials with octreotide reported in 1990 initiated a new approach. However, both trials were limited to a small number of excessively tall individuals who were treated for a relatively short period and without sufficient posttreatment follow-up. In both trials, a decrease in GH secretion during treatment was reported, accompanied by a striking reduction in growth velocity. But the deductions on predicting adult height are questionable, as are all predictions of this kind when concerning patients who are still growing. Moreover, the second report[2] reveals more inconsistencies than did the first one.*

It certainly must be pointed out that the use of octreotide for limiting growth is still at the stage of preliminary short-term trials. Larger studies, extended for years, are obviously needed. The experimental data show that SRIH analogue may have multiple effects. Those pertaining to the digestive tract and its secretions are probably of clinical importance, since SRIH has been used in several therapeutic approaches for several years. The limited data about possible side effects modifying the secretion of insulin and about other peptides that are regulated by somatostatin have not established adequately the safety of using a somatostatin analogue in the control of growth. However, since excessively tall stature is sometimes a social handicap, and the physiology and pathophysiology are important, the preliminary data reported are of significant interest.

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Growth Characteristics and Response to Growth Hormone Therapy in Patients With Hypochondroplasia: Genetic Linkage of the Insulin-Like Growth Factor 1 Gene at Chromosome 12q23 to the Disease in a Subgroup of These Patients

Mullis et al performed restriction enzyme analysis of the insulin-like growth factor 1 (IGF-1) gene of 20 hypochondroplastic white British children, 60 unrelated children with isolated growth hormone deficiency (IGHD), and 50 unrelated normal adults. Adult family members of the hypochondroplastic children were studied as well. All children with hypochondroplasia had normal growth hormone (GH) responses to insulin-induced hypoglycemia and plasma IGF-1 levels that were in the upper range of normal. Pretreatment height velocities were calculated for the hypochondroplastic and IGHD children for 1 year prior to treatment with recombinant human GH (rhGH) in doses between 18 and 32 U/m^2 per week. Since previous studies have shown that pretreatment height velocity is a predominant determinant of growth responses to rhGH in GHD children, the hypochondroplastic children were compared with a control group who had similar pretreatment height velocities. The groups also were matched for age, sex, and pubertal status. Sitting height and subischiatic leg length were determined.

Human lymphocyte DNA was isolated and digested with restriction enzymes *Hin*dIII and *Pvu* II, under conditions recommended by their suppliers. Southern blots of *Hin*dIII-digested DNA hybridized with a human IGF-1 cDNA probe showed nonpolymorphic fragments of 8.2 and 3.2 kb and a restriction fragment length polymorphism (RFLP) with alleles of 4.8 and 5.2 kb. *Pvu* II digests showed nonpolymorphic fragments of 8.4 and 2.5 kb and an RFLP with alleles of 4.7 and 5.1 kb. The frequencies of the heterozygous pattern (*Hin*dIII: 8.2, 5.2, 4.8, 3.2-kb fragments; *Pvu* II: 8.4, 5.1, 4.7, 2.5-kb fragments) in IGHD children and controls were 22% and 20% respectively, in contrast to 45% in the children with hypochondroplasia ($P<0.05$). No individuals were homozygous for the 5.2-kb *Hin*dIII/ 5.1-kb *Pvu* II allele. The children with hypochondroplasia could be subdivided into 2 groups according to the IGF-1 RFLP alleles they possessed.

The heterozygous group (*Hind*III: 5.2, 4.8 kb; *Pvu* II: 5.1, 4.7 kb) included 4 girls and 5 boys. The second group was homozygous (*Hind*III: 4.8, 4.8 kb; *Pvu* II: 4.7, 4.7-kb), and included 6 girls and 5 boys. These 2 groups of children did not differ in pretreatment height velocity standard deviation scores (SDS) for chronologic age, sitting height, or subischiatic leg length. However, following 1 year of rhGH treatment, the heterozygous group had a proportionate increase in back and leg length while the homozygous children had a disproportionate increase in back length with respect to leg length (P=.009). Five of the 7 families whose 9 children were heterozygous for the IGF-1 allele were studied. Those who possessed the 5.2-kb *Hind*III and 5.1-kb *Pvu* II alleles were slightly disproportionate and significantly shorter than parents and adult relatives without these alleles (P<0.005). Parents of affected homozygous children did not present with body disproportion and were of normal stature. The authors point out that the data suggest that there are 2 subgroups of children with common features of hypochondroplasia but with differences in their response to rhGH. They further state that the data indicate that a gene involved in a form of short stature with hypochondroplasia characterized by proportionate response to rhGH is linked to the IGF-1 gene locus at chromosome 12q23. They conclude that this polymorphism itself is not responsible for the hypochondroplasia since both sets of alleles are present in the normal population and since some of the parents of the affected homozygous children were heterozygous and of normal stature.

Mullis P, et al. *Clin Endocrinol* 1991; 34: 265-274.

Editor's comments: *This is an interesting study that presents fascinating data concerning the response of children with hypochondroplasia to rhGH. It is an excellent example of geneticists and endocrinologists working in tandem to understand the etiology of the heterogenous disease entities that may affect human growth.*

William L. Clarke, MD

Abnormal Growth Patterns and Adult Short Stature in 115 Long-Term Survivors of Childhood Leukemia

Schriock et al evaluated final height in 115 long-term survivors of acute lymphoblastic leukemia (ALL) treated at St. Jude's Children's Research Hospital during the years from 1967 to 1975. Subjects with trisomy 21, central nervous system (CNS) leukemia at diagnosis, or those older than 12 years of age at diagnosis were excluded from the analysis as were children who had not completed growth. None of the 115 patients had been treated with growth hormone (GH) and all experienced spontaneous puberty. A variety of chemotherapeutic protocols were utilized, although all patients received induction chemotherapy with prednisolone, vincristine, daunorubicin, and/or asparaginase. CNS prophylaxis consisted of 2,400 cGy cranial irradiation plus 5 concomitant doses of intrathecal methotrexate or 2,400 cGy craniospinal irradiation alone. Patients' heights were measured at diagnosis and at least annually utilizing a stadiometer. Heights were expressed as standard deviation scores (SDS). The final cohort consisted of 39 males and 76 females who had been followed for a mean of 13.8 ± 2.1 years since diagnosis.

Significant retardation was observed in height SDS from diagnosis to the completion of chemotherapy (P<.0001) and from the end of therapy to the last evaluation (P<.0001). Heights at diagnosis were >1 SD below population norms for 19% and >2 SD for 2%. At final evaluation, 74% of these patients had SDS ≤1 SD, and 37% had SDS ≥2 SD. Chemotherapeutic regimens did not appear to have differential effects on the findings; however, height SDSs were significantly different for those receiving cranial versus craniospinal irradiation. Six patients in the craniospinal group did not receive prophylactic irradiation until chemotherapy had been completed. Despite their growth decrement during chemotherapy (P<0.03), they had no significant overall change in final height SDS. Height SDS had decreased at the end of chemotherapy in 90% of children treated with cranial irradiation, and 30% had final height scores of ≥2 SD below population means. Changes in height SDS were correlated with age at diagnosis for the patients who received cranial irradiation. Growth retardation was most prominent in those with early onset disease. In addition, girls whose disease was diagnosed before age 8 had significantly greater decreases in height SDS after chemotherapy than those who were older at diagnosis.

The authors state that the median change in height SDS from diagnosis to the last evaluation was -1.5, corresponding to a mean height decrement of 9.1 cm. Their data contrast with that of other studies, which predict minimal effects on adult height in survivors of childhood leukemia. The authors state that this may be due to the failure of other investigators to follow patients until growth was complete. They further note that there have been changes in CNS prophylaxis over the last few years, including a reduction in total cranial irradiation and the elimination of spinal irradiation.

Schriock E, et al. *J Clin Oncol* 1991; 9: 400-405.

Editor's comments: *This well-conducted study demonstrates separate effects of chemotherapy and irradiation on final height in long-term survivors of childhood ALL. It is particularly interesting because only children who were younger than 12 years of age at diagnosis were studied, thus eliminating potentially minimal changes in height decrements that might be observed in pubertal children. GH evaluations were not reported for any of the subjects; thus, it is not known whether any had permanent loss of GH secretion. It is noteworthy, however, that all patients entered spontaneous puberty. It is hoped that future prospective studies will include the determination of GH secretion as well as insulin-like growth factor 1 levels, so that these findings might be more fully explained. In addition, it will be interesting to evaluate the effect of omitting spinal irradiation and lowering cranial irradiation doses on final height in survivors of childhood ALL. Dr. Stephen Shalet will cover the entire topic of growth and treatment of cancer, particularly leukemia, in* GGH *Volume 8, Number 3.*

William L. Clarke, MD

MEETING CALENDAR

Jan 3-10, 1992 Intro to Molecular and Cellular Research, Pacific Grove, CA. Info: A Singer, The Endo Soc. Fax: 301-571-1869.

Apr 8-12, 1992 16th Training Course on Hormonal Assay Techniques, Rockville, MD. Info: A Singer, The Endo Soc. Fax: 301-571-1869.

May 4-7, 1992 Ann Mtg of the APS/SPR/APA, Baltimore Convention Ctr, Baltimore, MD. Info: APS/SPR/APA Program Office. Fax: 708-427-1305.

May 6-8, 1992 Ann Mtg of the LWPES, Baltimore, MD. Info: Dr GP August, LWPES, Children's Nat'l Med Ctr. Fax: 301-460-8846.

May 26-31, 1992 5th Int'l Conf on the Cell and Molecular Bio of Chlamydomonas, Pacific Grove, CA. Info: G. Witman, Worcester Foun Exp Biol. Fax: 301-530-7079.

June 11-14, 1992 7th Ann Mtg of the Assoc of ACT, Rochester, MN. Info: C.R. Schad, ACT. Phone: 507-284-2950.

June 18-23, 1992 52nd Ann Mtg of the ADA, San Antonio, TX. Info: Mtgs Dept, ADA. Fax: 703-836-7439.

June 24-27, 1992 74th Ann Mtg of the Endo Soc, San Antonio, TX. Info: A Singer, The Endo Soc, Fax: 301-571-1869.

July 12-15, 1992 24th Ann March of Dimes Clinical Genetics Conf, Stanford, CA. Info: Prof Svs Dept, March of Dimes Birth Defects Found. Tel: 914-428-7100.

Aug 30 - Sept 5, 1992 9th Int'l Congress of Endocrinology, Nice, France. Info: NICE 92, c/o SOCF1, 14 Rue Mandar, 75002 Paris, France.

Sept 7-10, 1992 31st Ann Mtg of the ESPE, Zaragoza, Spain. Info: Dr A Ferrandez-Longas, Endocrine Unit, Miguel Servet Children's Hosp, Paseo Isabel la Catolica 3, 50009 Zaragoza, Spain. Tel: 34-976-355-700.

Sept 10-12, 1992 Int'l Congress on Growth Hormone and Somatomedins During Lifespan, Milan, Italy. Info: Drs D Cocchi/V Locatelli, Dept of Pharm, Sch of Med, Univ of Milan, Via Vanvitelli, 32, 20129 Milan, Italy.

Oct 8-9, 1992 Int'l Symp on Growth '92 — 2 Decades of Experience in Growth, Santiago de Compostela, Spain. Dr. S Rossetti, Ares-Serono Symposia, Via Ravenna 8-00161 Rome, Italy. Fax: 39-6-44291324.

Oct 10-14, 1992 44th Postgrad Assembly of the Endo Soc, Boston, MA. Info: A Singer, the Endo Soc. Fax: 301-571-1869.

Nov 9-13, 1992 Ann Mtg of Am Soc of Hum Genetics, San Francisco, CA. Info: M Ryan, ASHG. Fax: 301-530-7079.

June 3-7, 1993 4th Joint Mtg of the ESPE/LWPES, San Francisco, CA. Info: Prof M Grumbach, Univ of CA Sch of Med. Fax: 415-476-4009.

June 9-12, 1993 75th Ann Mtg of the Endo Soc, Las Vegas, NV. Info: A Singer, The Endo Soc. Fax: 301-571-1869.

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